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The Hypothalamus, Intrinsic Connections and Outflow Pathways to the Endocrine System in Relation to the Control of Feeding and Metabolism

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THE HYPOTHALAMUS, INTRINSIC CONNECTIONS AND OUTFLOW PATHWAYS TO THE ENDOCRINE SYSTEM IN RELATION TO THE CONTROL OF FEEDING AND METABOLISM

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Abbreviations

A, AH, AHA, NHA	anterior hypothalamic area
ACB	accumbens nucleus
AHA, HI	amygdalohippocampal area
AIV	Ventral agranular insular cortex
AMB	ambiguus nucleus
AME, AM, ME, MA	medial amygdaloid nucleus
AP	area postrema
AR	arcuate nucleus
BL, ABL	basolateral amygdaloid nucleus
BM	basomedial amygdaloid nucleus
BST	bed nucleus of stria terminalis
CE, AC	central amygdaloid nucleus
CI, CAI, IC	internal capsule
DMH, DM	dorsomedial hypothalamic nucleus
EM	median eminence
F	fornix
FMP	medial forebrain bundle
IML	intermediolateral cell-group
LA, AL	lateral amygdaloid nucleus
LFU	lateral funiculus
LHA, LH	lateral hypothalamic area
LL	lateral lemniscus
LR	lateral reticular nucleus
LS	lateral septal area
MDD	dorsal reticular nucleus
MDV	ventral reticular nucleus
ML, LM	medial lemniscus
MPO	medial preoptic area
MT	mammillothalamic tract
N III, III	oculomotor nucleus
NTS	solitary tract nucleus
PAG, CG, SGC	periaqueductal gray
PF	perifornical area
PIT	pituitary
PL	prelimbic cortical area
PMD	dorsal premammillary nucleus
PMV	ventral premammillary nucleus
PP	peripeduncular area
PR	perirhinal cortical area
PV	paraventricular nucleus
PVM	magnocellular paraventricular nucleus
PVP	parvocellular paraventricular nucleus
RET	medullary reticular formation
RF	rhinal fissure
RGI	gigantocellular reticular formation
RPC	parvocellular reticular formation
S	subiculum
SI	substantia innominata
ST	stria terminalis
TO, OT	tractus opticus
TS	tractus solitarius
V III	third ventricle
VDB	nucleus vertical limb diagonal band
VFU	ventral funiculus
VMH, VM	ventromedial hypothalamic nucleus
X, 10	dorsal motor nucleus of the vagus
ZI	zona incerta
8	area 8 of Rexed
9	area 9 of Rexed
10	area 10 of Rexed
XII	hypoglossal nucleus

1. Introduction

1.1. GENERAL: THE POSITION OF THE HYPOTHALAMUS IN THE LIMBIC SYSTEM

In most general terms the function of the limbic system in the mammalian brain is to organize behavioral patterns that result in the most suitable way for survival of specimen

and species. As such the limbic system plays a key role in behaviors that are essential for the survival of the individual organism such as feeding, drinking, and defense or attack behavior, or for the survival of the species such as reproductive behavior and agonistic behaviors in territorial situations.

The 'limbic system' itself is a highly complex compound system of strongly interrelated structures in telencephalon, diencephalon and mesencephalon. Although some dispute exists concerning the precise definitions of the limbic system a more or less general consensus exists about its major components. At telencephalic levels the limbic cortical ring i.e. cingulate and parahippocampal cortices, hippocampal formation and amygdaloid body are considered as its major constituents. Although the prefrontal cortex is not always regarded as an integrated part of the limbic system, its functional influence and anatomical relations are so evidently clearly 'limbic' in nature that it will be implied here as well (Cotman and McGaugh, 1980). Basal forebrain structures like the septum and preoptic region are considered as part of the limbic circuit (Nieuwenhuys *et al.*, 1978), whereas in the diencephalon, the hypothalamus (including the mammillary bodies) are the limbic representatives. In the midbrain some cell groups in reticular formation and periventricular gray contribute to the limbic functions.

As a domain of complex integration, the limbic system is provided with a permanent body of information both from the internal and external environment. Likewise the limbic system can exert its influence via the two major effector channels of the brain: the true motor systems and the autonomic-neuroendocrine outflow. Specifically with respect to the overall input-output organization of the limbic system, we may mention here the position and importance of the hypothalamus. Speaking in general terms, the hypothalamus guards a part of the channels of entry and the majority of the outflow of information to and from the internal environment of the mammalian body. It makes the first overall selection of certain inputs and a major selection of output to and from the 'higher' integration centers of the limbic system. As such, the hypothalamus is not only a center of relay of information, but acts as a center of integration as well. Focussing such a large number of important functions in a structure of such moderate size leads to two easily discernable conclusions: its anatomy is complex and the effects of even minor damage are dramatic.

1.2. THE HYPOTHALAMUS AND THE CONTROL OF FEEDING

With respect to the role of the hypothalamus in the control of feeding and metabolism, it has been demonstrated in a large number of studies that even small lesions in various hypothalamic structures may result in either increased (hyperphagia) or decreased (hypo- or aphagia) food intake and/or changes in body weight. At the same time, however, input-, output- and interconnections of the hypothalamic structures involved, are yet far from entirely understood (Ricardo, 1983). Recent advances in neuroanatomical methodology, on the other hand, have widened our insights into the substrate for complex behavioral functions of the hypothalamus.

Food intake as a behavioral act—apart from being a pure motor event—appears to be a very complex phenomenon in terms of its appearance in time, and relation to 'hunger' and 'satiety', meal size, meal duration, onset and end of the intake period, etc. The act of feeding, however, can also simply be considered as an act of motor activity to match the needs for the three major nutrients for cellular metabolism: glucose, free fatty acids and amino acids present in the ingested food as carbohydrates, fat and proteins. In this definition, feeding may be translated in terms of maintenance of the organism's composition of the three major classes of food components. Such an interpretation, moreover, has enabled physiologists to express and measure the event of feeding in quantifiable and qualifiable processing of chemical compounds. The process of feeding actually serves the living cell with quantities or concentrations of glucose, free fatty acids and amino acids that are maintained within certain limits to meet the cell's metabolic demands.

This process of stabilization of plasma fuel substrate levels—or fuel substrate homeostasis—can now be considered as one of the dominant metabolic control functions of the hypothalamus.

In the present survey we will summarize our data on the structural organization of the hypothalamic substrate that underlies the metabolic regulatory function as studied in the rat. We will focus on the pathways mediating the hypothalamic signals that underlie the hormonal release mechanisms responsible for glucose homeostasis, and how these hypothalamic pathways are embedded in the limbic system circuitry.

1.3. THE HYPOTHALAMUS AND METABOLIC REGULATORY FUNCTIONS

In general, the level of glucose in the blood is maintained physiologically within certain minimum and maximum values by a finely tuned balance of hormones of the endocrine pancreas: primarily insulin and glucagon. A third hormone, somatostatin, seems to be involved in tuning insulin and glucagon secretion by its paracrine action.

Specifically the release of insulin and glucagon appear to be under profound influence of the hypothalamus. Electrical or chemical stimulation of the lateral hypothalamic area (LHA) results in increased levels of insulin, whereas glucagon levels appear to be unchanged (De Jong *et al.*, 1977; Frohman and Bernardis, 1971; Shimazu and Ishikawa, 1981; Steffens, 1981; Steffens *et al.*, 1984a, b). These effects on insulin secretion are blocked by atropine which indicates the parasympathetic autonomic nature of these control mechanisms (Steffens, 1981). Indeed it was observed that electrical stimulation of the vagal nerve causes sharp rises in insulin levels (Kaneto *et al.*, 1967). Either electrical or chemical stimulation of the ventromedial hypothalamic nucleus (VMH), on the other hand, elicits a rise in plasma glucagon levels mediated by sympathetic components of the autonomic nervous system that are blocked by the ganglion blocker hexamethonium (Berthoud and Jeanrenaud, 1979; Steffens, 1981). The same effects on glucagon levels can be achieved by applying electrical current to the splanchnic nerve (Bloom *et al.*, 1973; Bloom and Edwards, 1975). The role of the dorsomedial hypothalamic nucleus (DMH) in glucose homeostasis is far less clear. Stimulation studies performed on the DMH indicate rises of glucose levels, however, with no clear change in insulin. It has recently been reported that DMH electrical stimulation leads to increased levels of peripheral norepinephrine (NE), which may be responsible for the observed increase of glucose (Halter *et al.*, 1984). Lesion studies, moreover, show that the DMH exerts a complex influence on feeding and in particular on body weight (Bernardis, 1973, 1985; Bernardis and Bellinger, 1984; Dalton *et al.*, 1981).

Additional evidence for the impact of VMH, LHA and DMH on pancreatic hormone release via neural autonomic pathways is given by the elegant work of Yoshimatsu *et al.* (1984) (Fig. 1). These authors described the effects of bilateral lesions of food intake-related hypothalamic nuclei on the activity of splanchnic and vagal nerves to the pancreas. In all cases, these authors observed simultaneous changes in firing rates in the sympathetic and parasympathetic branches innervating the pancreas as exemplified in Fig. 1. It can clearly be seen in this case that lesions of the lateral hypothalamic area had a profound effect on the activity rate of the vagal nerve and some minor inhibitory or excitatory effects on splanchnic nerve activity, depending on the precise position of the lesion.

1.4. THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS, METABOLIC REGULATION AND THE ADRENAL HORMONES

One of the most powerful stimulation sites of the mammalian hypothalamus by which feeding can be influenced is the paraventricular nucleus (PVN). Although our knowledge on the effects of PVN stimulation on serum levels of pancreatic hormones and catecholamines is still scarce, it has been shown that NE stimulation of PVN leads to a significant increase of food intake (Leibowitz, 1978; Leibowitz *et al.*, 1981; Weiss and Leibowitz, 1983). Preliminary data on PVN stimulation with NE, which we currently perform in our laboratory, show a considerable hyperglycemia and a simultaneous increase of insulin. Combined hyperinsulinemia and hyperglycemia indicates a strong interference by epinephrine, apparently as a result of PVN stimulation of the adrenal glands. As we have indicated above, there is an interaction between hormones released from the adrenal cortex

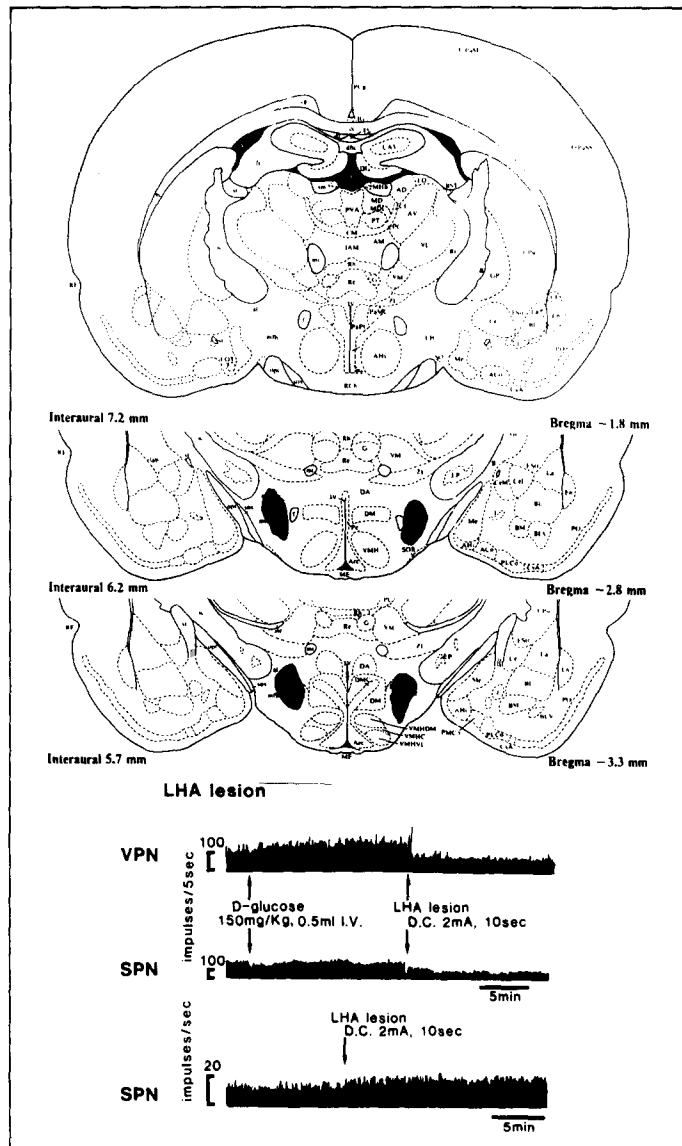


FIG. 1. Transverse sections through the rat hypothalamus in which is indicated the position and size of bilateral lesions in the lateral hypothalamic area (black spots). In the bottom part of the figure three recordings are given of the vagal pancreatic nerve (VPN) and splanchnic pancreatic nerve (SPN) before and after the time of LHA lesion. One can observe a clear drop in vagal nerve activity and either an increase or decrease in splanchnic nerve activity dependent on the intravenous glucose-load. (Modified with the author's permission from Yoshimatsu *et al.*, 1984.)

and medulla, and the hormone released from the pancreas (Samols and Weir, 1979). From an anatomical point of view, the PVN, via its neuroendocrine and autonomic connections (Swanson and Sawchenko, 1983), is in a position to control the release of adrenal hormones. It is thus tempting to hypothesize that the effects of the organism's emotional state on feeding—and possibly also on other limbic behaviors—are carried out by control mechanisms that are dominated by the PVN. The anatomical analysis that we have carried out, provides an increasing amount of data to support this assumption. It appears that the PVN, by means of its extensive network of neuroendocrine and autonomic connections, is superimposed on the feeding control substrate. This way the emotional state of the organism—and possibly also stress and other arousals—may interfere with feeding and metabolic regulatory mechanisms.

In this review we will present anatomical data on the nervous pathways that underlie

the physiological processes of hypothalamic influence on feeding and metabolic homeostatic mechanisms. This functional approach to an anatomical analysis does not mean that the anatomical circuitry that we will describe serves exclusively in functions of feeding and control of metabolism. As a matter of fact only limited numbers of neurons in e.g. VMH or LHA have been proved to be directly related to feeding rhythms (T. Ono, personal communication). So we will conclude that the pathways we describe may be considered as more general autonomic or neuroendocrine output connections. The limitation of our interpretation to feeding and metabolic control, however, will enable us to study the functional significance of anatomical data in a physiological paradigm or, in other terms, to study anatomy in a functional perspective.

2. Neuroanatomical Methods

2.1. TRACT-TRACING METHODS IN NEUROANATOMY

Reviewing, in brief, the development of our knowledge on the anatomical nature of the central nervous system pathways, it is clear that great advances were made by the introduction of the so-called tract-tracing methods. The first major step forward during the post-war decades was the introduction of the degeneration methods, of which the Nauta-Gygax and Fink-Heimer procedures are the most well-known. These methods are based on the principle of selectively staining the degeneration products of experimentally damaged nerve cell processes. The use of these methods in recent years has become overshadowed by the disadvantages inherent to these methods. The degeneration methods are all based on destruction of living material and thus provide artifacts from which a deduction should be made, carrying all the risks of misinterpretation. Secondly, the degeneration methods in many cases suffered from unavoidable flaws such as the fiber-of-passage problem, i.e. causing damage to passing fibers that are irrelevant to the structure under study.

2.1.1. *Intra-axonal transport. Retrograde transport methods*

Many of these problems were overcome—and hence followed by a spectacular revival of research interest—by the introduction of the intra-axonal transport methods. All these methods are based on the principle of transport of exogenous compounds by the living nerve cell, either from synapse to soma (retrograde transport) or from soma to presynaptic bouton (anterograde transport). The most commonly known and still widely used is the method of retrograde transport of horseradish peroxidase (HRP). With the HRP procedure, the exogenous HRP is locally applied to a brain structure, after which the presynaptic boutons take up the tracer and transport it back to the soma, thus displaying the sources of afferents to the area of injection. From its introduction (LaVail and LaVail, 1972), the HRP method underwent continuous improvement mainly with respect to the methods of injection and the sensitivity of techniques to visualize and stabilize the tracer compound and its reaction products. Furthermore, a large number of compounds were developed that could replace HRP in retrograde transport experiments, such as HRP conjugated with wheat-germ agglutinin (WGA-HRP) and various fluorescent tracers such as nuclear yellow, true blue, granular blue etc. (Kuypers *et al.*, 1977). The advantages of the retrograde transport methods can be summarized as giving much information on nervous connectivity in a short period of time. This information, however, is limited in the sense that the retrograde transport methods usually give only information on afferents to the structure studied, but no or only limited data on nerve cell projections.

2.1.2. *Anterograde transport methods*

Efferent connections, on the other hand, can more reliably be studied with anterograde transport methods employing exogenous non-labeled or radioactively labeled compounds that follow the nerve cell's cytoplasm flow from soma to terminal presynaptic bouton. Until

recently the most widely applied anterograde transport method is the autoradiographic tracing of tritiated aminoacids. With this method tritiated aminoacids—usually leucine or proline—are applied to brain nuclei whose cell bodies absorb these aminoacids that are subsequently synthesized to proteins. The labeled proteins are transported, via slow intra-axonal cytoplasmic flow, to the neurites terminal structures. After the appropriate survival time of approximately one week, the transported radioactive tracer is visualized by means of photographic film emulsions applied over the brain sections. After an exposure period, a specific sensitivity to the tracers' radioactive emission is displayed. A disadvantage of this method is, however, that the autoradiograph gives an indirect reflection of the labeled structure and not the actual structure itself. For that reason one cannot with certainty discriminate between labeling of fibers and labeling of terminal presynaptic boutons, i.e. the site of synaptic transfer of information. Moreover, the autoradiographic method, as we will see, is not suitable for investigating short distance connections, which is a major drawback when studying intrahypothalamic circuitry.

Most of these disadvantages were overcome by a recently developed alternative for tracing neuronal efferents by means of the lectin *Phaseolus vulgaris* leuco-agglutinin (PHA-L) (Gerfen and Sawchenko, 1984). With this method, PHA-L, after introduction into the nervous system, is actively taken up by the neural soma membrane via pinocytosis. After entry into the cell body, the PHA-L is readily transported throughout the entire axonal system, filling the fibers up to the finest branching and their presynaptic terminations. After filling the efferent nerve fibers, the PHA-L is visualized by means of immunocytochemical procedures employing specific antibodies against PHA-L followed by Sternberger's PAP method (Sternberger, 1974). This method yields dark-brown stained cellbodies including dendrites and the entirely stained axons carrying the budlike presynaptic boutons, either terminal or 'en passant' (Gerfen and Sawchenko, 1984; Ter Horst *et al.*, 1984; Wouterlood and Groenewegen, 1985).

2.2. INJECTION PROCEDURES. IONTOPHORETIC VS MECHANICAL TRACER INJECTION

A major problem related to all experimental tract-tracing techniques is the control of the size of the tracer injection. The dimensions of the tracer injection determine the tracer uptake-area and thus marks the interpretation of the connectivity found in terms of site of origin of tracer-labeled structures. Without going into detail of the problems related to tracer injections, it has been our experience that iontophoretic delivery methods produce the smallest possible injection sites with a reasonable degree of reproducibility. Iontophoresis via glass micropipettes results in delivery of molecules instead of injection of relatively large volumes in a medium like the brain that contains very limited extracellular space. Consequently, tissue damage with iontophoretic injection procedures is minimized as compared to mechanical ways of tracer delivery.

In all the experiments carried out in our department that are described in the present review, the tracer (HRP, WGA-HRP, ^3H -leucine and PHA-L) was injected into the CNS by means of iontophoretic procedures. Usually the tracer is dissolved in a buffered carrier solution and brought into the brain via bevelled glass micropipettes with tip diameters between 10–20 μm . The pipette is connected to a constant-current source that generates the driving force for iontophoresis. Current intensity, polarity and duration are dependent on tracer molecule charge, osmolality of the solution and tracer concentration and varied between 0.5–5 μA for 10–30 min. For a detailed description of the HRP procedure followed in our experiments we refer to Ter Horst *et al.* (1984) and Mesulam (1978). The procedure of Rye *et al.* (1984) was followed in our more recent experiments. The method of autoradiographic tracing including injection parameters is briefly described in Luiten *et al.* (1985a). For a more extensive account we may refer to Edwards and Hendrickson (1981). Literature on the recently introduced PHA-L method is much more limited. The original paper of Gerfen and Sawchenko (1984) provides excellent information, more specifically on fluorescent visualization. Demonstration of PHA-L labeling by means of

the PAP method is described by Ter Horst *et al.* (1984a), whereas the procedure applied for electron microscopic purposes is given in a report by Wouterlood and Groenewegen (1985).

3. The Hypothalamus and its Relation to 'Higher' Limbic Structures

Assuming an output function for the hypothalamus in the limbic system organization, it will be obvious that the hypothalamus must receive a considerable descending forebrain input from higher limbic structures. A general picture of such input can be obtained from the retrograde labeling of nerve cell bodies following injections of HRP into—in our case—the feeding-related nuclei of the hypothalamus: the VMH, DMH, and LHA.

3.1. LIMBIC INPUT TO THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS

Various authors have reported on the input sources to the hypothalamus, both from descending and ascending origin and studied with a variety of tract-tracing methods (Kita and Oomura, 1982a, b; Luiten and Room, 1980; Luiten *et al.*, 1983; McBride and Sutin, 1977; Ricardo, 1983).

We will describe such inputs by the results of labeling after injections of retrograde tracers in the lateral and ventromedial hypothalamic nuclei. Figure 2 shows a pattern of retrogradely labeled neurons that, as a whole, constitute a mainly descending limbic input to the VMH. This pattern is largely consistent with the previous description of data presented in analogous investigations by Luiten and Room (1980), Kita and Oomura (1982a, b), McBride and Sutin (1977) and Ricardo (1983). At most anterior levels, a considerable number of cells became labeled in the lateral septal nuclei, itself a major target for outgoing hippocampal efferents (Swanson and Cowan, 1977; Meibach and Siegel, 1977) and ascending monoaminergic input (Luiten *et al.*, 1982). Further posterior labeling occurs in the bed nucleus of the stria terminalis, in the supraoptic nucleus and in the medial preoptic area. In the anterior levels of the hypothalamus, a source of VMH afferents is found in the parvocellular part of the paraventricular nucleus, the anterior hypothalamic nucleus and in the bottom structures of the floor of the hypothalamus, equivalent to medial and lateral aspects of the retrochiasmatic area. At the level of injection, retrograde labeling occurs in the dorsomedial nucleus of the hypothalamus and more conspicuously in the amygdaloid body, from which the medial amygdaloid nucleus is always the most heavily labeled. More posterior amygdaloid labeling is present in the amygdalo-hippocampal nucleus, that appears continuous with rich labeling in the ventral subiculum of the hippocampal formation (Fig. 2E). In some experiments with HRP injections into the VMH, some sparse labeling occurred in the ventral CA₁ field of the Ammon's horn. Labeling of ascending input to the hypothalamus, e.g. from brachium conjunctivum, dorsal tegmentum and raphe structures, have been described extensively before (see review of Ricardo, 1983) and will not be dealt with further in the scope of this paper.

The pattern of input connections described above may be summarized in the sense that the VMH is the recipient of a considerable input from two dominant limbic structures: amygdaloid body and hippocampal formation. Both structures may relay information to the VMH via direct and indirect connections. The hippocampal formation, by way of ventral subicular projections, has direct access to the VMH (see also Swanson and Cowan, 1977), but a major, though indirect, connection is likely to be formed by the extensive hippocampal output to the lateral septum and from there to the VMH.

The amygdala likewise has two ways of information transfer to the VMH. One direct connection via the so-called ventral amygdalofugal pathway (see Section 3.4), and again a larger indirect channel via the bed nucleus of the stria terminalis.

3.2. LIMBIC INPUT TO THE LATERAL HYPOTHALAMIC AREA

The descending limbic inputs to the lateral hypothalamic area (LHA) in HRP experiments show a rather different pattern as compared with the input to the VMH. HRP

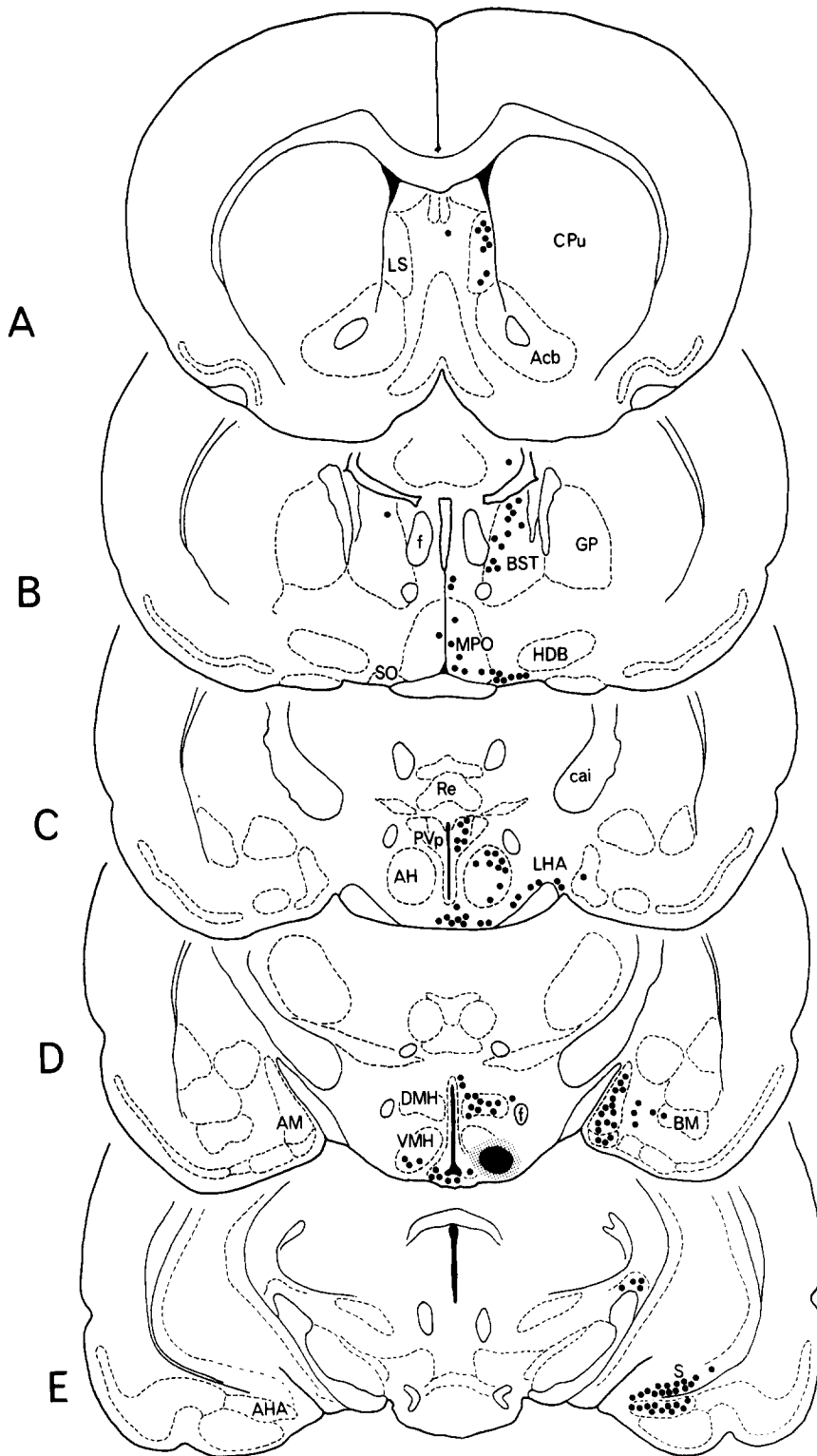


FIG. 2. Series of transverse sections from anterior (A) to posterior (E) in which is indicated the appearance of retrogradely labeled somata as a result of a horseradish peroxidase injection in the more ventral aspects of the ventromedial hypothalamic nucleus.

injections in the LHA resulted in labeling of large numbers of somata in the medial and orbital regions of the prefrontal cortex and in the prelimbic cortex (Fig. 3A). In particular labeling in the latter limbic cortical region is very numerous. At the same level, a large amount of labeled cells appears in the nucleus accumbens. More caudally, again we

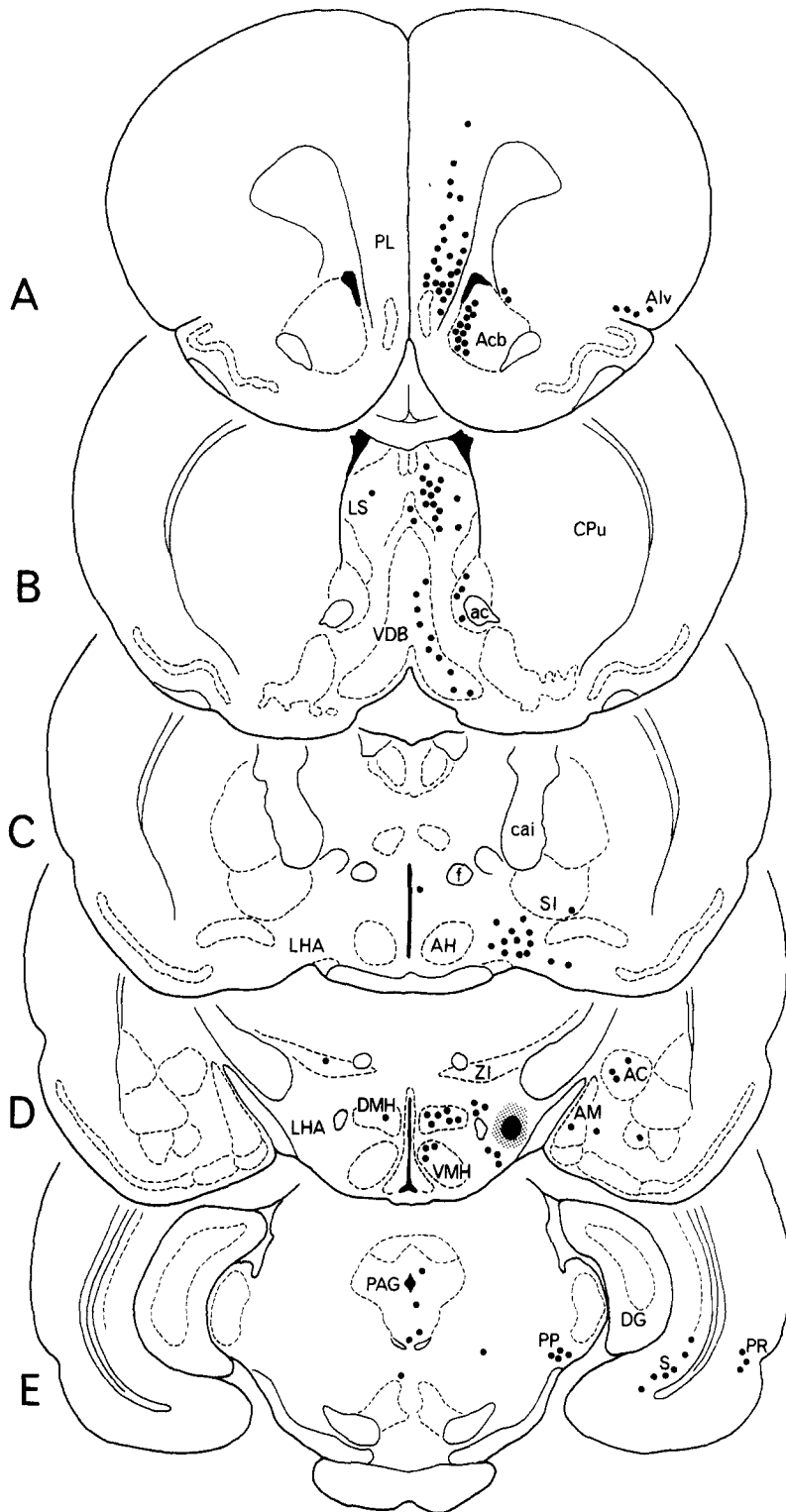


FIG. 3. Transverse sections through the rat brain in which is indicated the retrograde labeling of nerve cell bodies (black dots) following an injection of HRP in the lateral hypothalamic area.

observed considerable labeling in the lateral septal area, but also in the vertical limb nucleus of the diagonal band of Broca (Fig. 3B), whereas labeling in the bed nucleus of the stria terminalis is sparse. In the hypothalamus the sources of LHA afferents are limited to the dorsomedial nucleus, some cells in the dorsal aspects of the VMH and in various levels of the medial forebrain column. Conspicuously, the amygdaloid body is only a minor contributor of input to the lateral hypothalamus as is the subiculum of the hippocampal formation (Ono *et al.*, 1985). Some scarce labeling was observed in the perirhinal area of the parahippocampal cortex.

So in summary, the descending limbic input to the LHA is dominated by the anterior limbic cortical areas and the nucleus accumbens. There is a reasonably well-developed way of entry of hippocampal information via the lateral septal pathway, although direct hippocampal input to the LHA appears to be limited. The amygdaloid body, in contrast to its impressive connections with the medial hypothalamus, has only limited projections to the lateral hypothalamic area both directly or indirectly via the stria terminalis and its bed nucleus. The amygdaloid input to the LHA, however, although scarce, should not be underestimated, since particularly in food intake control their interconnections appear to be of considerable functional significance (Ono *et al.*, 1981; Oomura and Ono, 1982).

From the HRP experiments described above, it became clear that the amygdaloid body, at least with respect to its role as an input source to the VMH, maintains a dominant position. Furthermore the amygdaloid body and hypothalamus have in common that lesions in both structures appear to result in dramatic changes in feeding related behaviors. This led us to study amygdaloid input organization to the VMH, DMH and LHA in more detail (Luiten *et al.*, 1983; Ono *et al.*, 1985), in order to establish a possible link between an anatomical amygdalo-hypothalamic organization and a functional specificity. Anatomically it became evident that there exists a differentiation in amygdaloid afferents to various anterior-posterior and dorso-ventral divisions of the VMH, DMH and LHA. This topographic pattern was shown by extremely small HRP injections in subdivisions of the three hypothalamic nuclei mentioned. Although the pattern of amygdaloid labeling is complex, it is clear that the medial amygdaloid nucleus and amygdalo-hippocampal area are the major donors of input to the VMH, although the central, lateral, basomedial and basolateral amygdaloid nuclei also supply efferents to the VMH. A general conclusion is that posterior cell groups of the amygdala contribute efferents to more ventral subdivisions of the VMH. The more anterior parts of the amygdaloid nuclei project to more dorsal aspects of the VMH, and to the posterior region of the LHA. The central amygdaloid nucleus appears to be the only amygdaloid input source of the DMH (Ono *et al.*, 1985).

In the HRP experiments that are described above, both from our own studies and from others, an overall picture has been achieved of input sources afferent to the hypothalamus. The HRP method, however, does not permit us to draw conclusions as to the fine nature of termination of the input pathways within the hypothalamic target structures. Such information requires the application of so-called anterograde transport methods such as the ^3H -leucine autoradiographic tracing method or the more recently developed *Phaseolus vulgaris* leuco-agglutinin immunocytochemical tracing (see Section 2.1.2). These methods have been extensively applied in particular to septal, hippocampal and amygdaloid efferents and their pattern of hypothalamic termination has been described in more or less greater detail (Krettek and Price, 1978; Luiten *et al.*, 1985a; Meibach and Siegel, 1977; Swanson and Cowan, 1977, 1979).

For two of the input sources to the hypothalamus, i.e. the prefrontal cortex and the medial amygdaloid nuclei, we will describe here their pattern of hypothalamic innervation as obtained with anterograde tracing methods.

3.3. PREFRONTAL PROJECTIONS TO THE HYPOTHALAMUS

In retrograde tracer experiments (Sections 3.1 and 3.2), it was demonstrated that the prefrontal cortex, and in particular the prelimbic cortex (PL), is a donor of efferents to

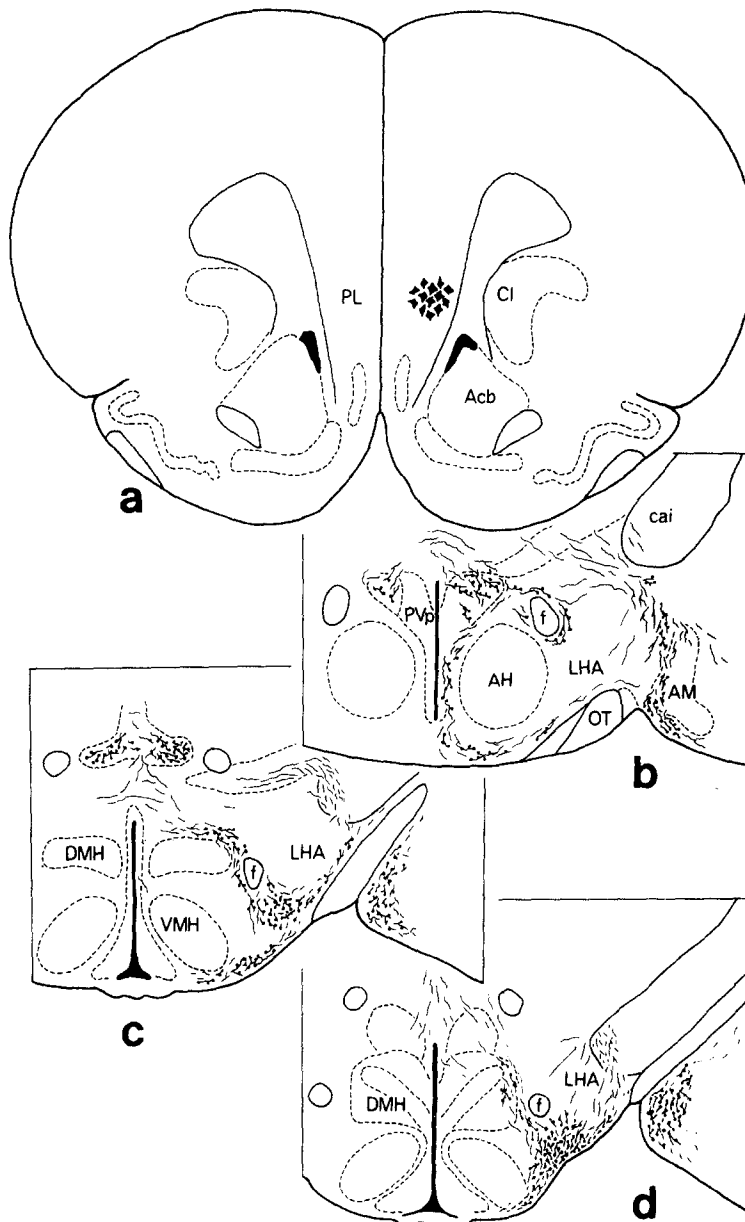


FIG. 4. Series of transverse sections with an injection of *Phaseolus vulgaris* leuco-agglutinin in the prelimbic cortex (PL) in (a). The projections of the PL to the hypothalamus are depicted in (b)–(d) as anterogradely labeled axons and terminal boutons, that are drawn as tiny buds, that are richly present in the lateral hypothalamic area.

the lateral hypothalamic area. Studying PL efferents with the PHA-L methods gave us the opportunity to establish in detail the hypothalamic site of termination of PL projections. This was clearly demonstrated by a rich pattern of labeled presynaptic boutons both of the terminal and en-passant type, predominantly in the more ventral aspects of the posterior parts of the lateral hypothalamic area (Fig. 4c, d). Furthermore, some terminal labeling occurred in the anterior hypothalamus, the parvocellular paraventricular nucleus, the perifornical area and in the periventricular grey. No projections could be observed to the ventromedial hypothalamic nucleus.

Others, who studied prelimbic and infralimbic efferents by anterograde tracing in cat, achieved comparable results (Room *et al.*, 1985). Together with the retrograde transport data, the existence of a prelimbic input to the lateral hypothalamic area is now firmly established.

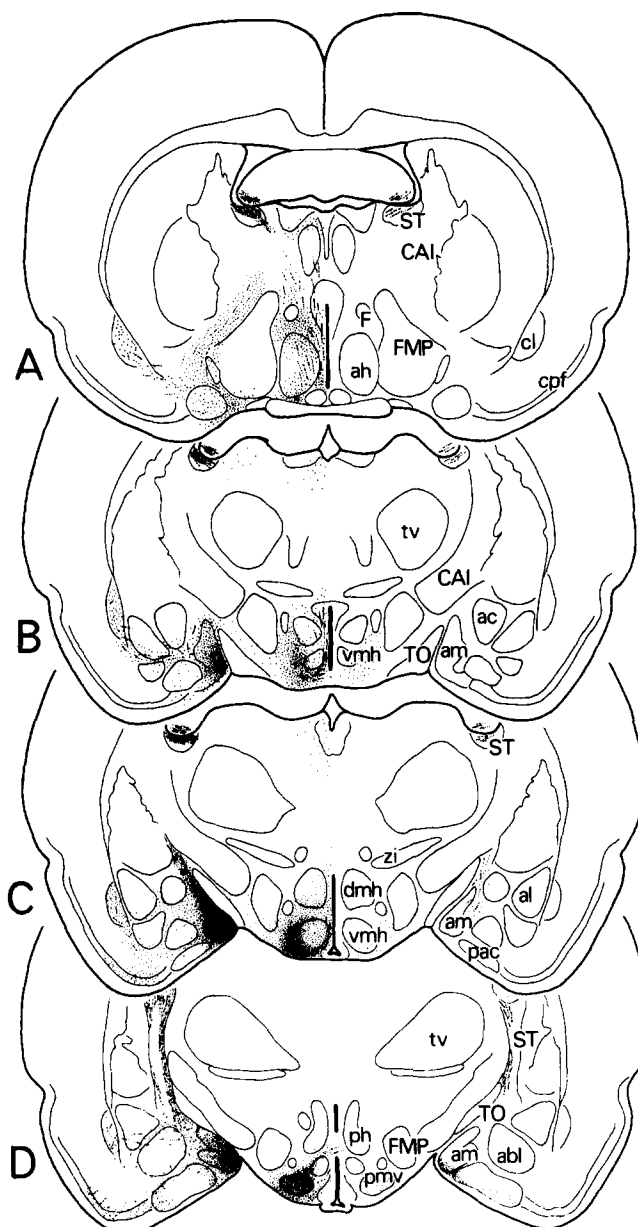


FIG. 5. The pattern of anterogradely labeled projections from amygdala to hypothalamus following a tritiated leucine injection in the medial amygdaloid nucleus (am) (c). Note the major target labeling in the ventromedial hypothalamic nucleus (vmh) and ventral premammillary nucleus (pmv).

3.4. AMYGDALOID PROJECTIONS TO THE HYPOTHALAMUS

The pattern of amygdaloid projections to the hypothalamus has been studied in considerable detail by various authors (Krettek and Price, 1978; Luiten *et al.*, 1985a). The hypothalamic projection of the medial amygdaloid nucleus, one of the more prominent sources of amygdaloid input to the hypothalamus, is illustrated in Fig. 5 by the results of a ^3H -leucine injection and was confirmed by PHA-L experiments (Fig. 6). The labeled efferents aimed at the hypothalamus all run via the stria terminalis and none by the ventral amygdalofugal pathway that apparently carries only amygdalopetal connections. The labeled stria fibers, for a small part, seem to terminate in the dorsal anterior hypothalamic nucleus (AH) and the AH surrounding neuropil. More caudally at the levels of the VMH, a dense pattern of labeling can be observed. It appeared that the descending fibers run in a medial, periventricular position then bend ventro-laterally around the VMH and

terminate densely in the ventrolateral core of the VMH and within the fiber shell that characteristically surrounds this nucleus (Millhouse, 1973) (Fig. 5C). At posterior hypothalamic levels, a very strong and also very circumscribed medial amygdala projection can be seen in the ventral premammillary nucleus (PMV in Fig. 5D and Fig. 6B,C). In Section 4.6 we will see that this highly specific projection to the PMV may be an important relay for an amygdaloid entry to the paraventricular hypothalamic nucleus, to which the limbic structures do not seem to have strong direct connections.

The amygdalo-hippocampal area, which is the second major donor of amygdaloid input to the hypothalamus, sends fibers to the perinuclear fiber shell of the VMH and also to the PMV (Krettek and Price, 1977; Ono *et al.*, 1985). The central amygdaloid nucleus was described as being one of the few amygdala sources of input to the dorsomedial hypothalamic nucleus. Except for the amygdalo-hippocampal area, basically all other amygdaloid cell groups do contribute efferents, although moderately, to the lateral hypothalamic area.

3.5. PROJECTIONS OF THE HIPPOCAMPAL FORMATION TO THE HYPOTHALAMUS

Several authors have reported on a direct input from the hippocampal formation to the hypothalamus, as studied by anterograde tracing methods (Krettek and Price, 1977; Meibach and Siegel, 1977; Swanson and Cowan, 1977). In line with the retrograde tracer studies that we described above, it may be concluded that the ventral subiculum is the sole source of direct hippocampal projections to the hypothalamus. These fibers all run via the fimbria-fornix pathway through the septum and preoptic region and terminate in the perinuclear fiber shell of the VMH, with some minor contribution to the medial parts of the lateral hypothalamic area (Krettek and Price, 1977; Swanson and Cowan, 1977).

One has to take into account, however, that the hippocampal formation as a whole has a main outflow projection to the lateral septum (Swanson and Cowan, 1977; Meibach and Siegel, 1977), from where projections reach, in particular, the lateral and dorsomedial hypothalamic nuclei (Luiten and Room, 1980; Swanson and Cowan, 1979). So in summary, we may conclude that the hippocampal formation, by way of its subicular output, has direct access to the shell of the ventromedial nucleus and via the lateral septum may reach the dorsomedial and lateral hypothalamus. There is no evidence for direct pathways to the paraventricular hypothalamic nucleus.

3.6. FOREBRAIN LIMBIC INPUT TO THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS

The investigations we have described so far failed to reveal any significant input to the PVN complex from the telencephalic limbic areas, apart from some prelimbic cortical afferents. From the point of view of descending limbic input, the PVN apparently takes a special and peculiar position. The most thorough analysis of afferents to the paraventricular nucleus is from Sawchenko and Swanson (1983), who described labeling in the dorsolateral septum and bed nucleus of the stria terminalis following retrograde tracer injections in the paraventricular nucleus. Additional, and also well-developed, projection pathways appear to originate from the subfornical organ (SFO) and, as we know from our own observations, from the organum vasculosum lamina terminalis (OVLT). The possible significance of the input from these circumventricular organs will be discussed in Section 4.4.

3.7. NORADRENERGIC INPUT TO THE HYPOTHALAMUS

Although in the present survey we have focussed our attention on the hypothalamus as a limbic output center, and thus on the position of the hypothalamus in the descending limbic outflow, we should also mention here at least one source of ascending input that has been demonstrated as being of critical importance in the regulation of feeding and metabolism. It has now been well documented that noradrenergic fibers to the hypo-

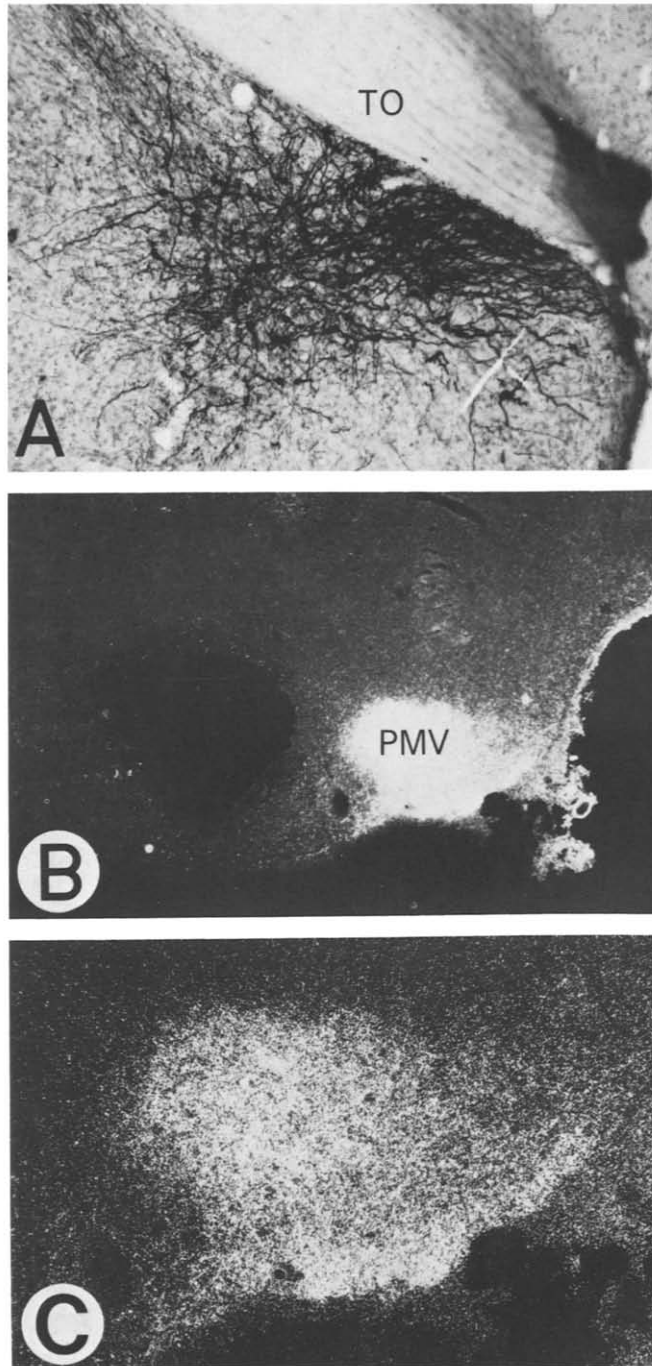


FIG. 6. (A) Photomicrograph of a *Phaseolus vulgaris* leuco-agglutinin injection in the medial amygdaloid nucleus. (B) and (C): Darkfield photomicrographs in survey (B) and detail (C) of terminal labeling in the ventral premammillary nucleus following a tritiated leucine injection in the medial amygdaloid nucleus. Compare (B) and (C) with drawing (D) of Fig. 5.

thalamus that originate in noradrenergic cell groups A1 and A2 in the lower medulla, via the ventral noradrenergic bundle, have a moderate projection to the lateral hypothalamic area and a rather dense projection to the paraventricular and dorsomedial hypothalamic nuclei (Jacobowitz and Palkovits, 1974; Sawchenko and Swanson, 1981; Swanson and Hartman, 1980; Takagi *et al.*, 1980). We may emphasize this noradrenergic input because of the relation between NE turnover and the act of feeding (Van der Gugten and Slangen, 1977) and the profound effects on feeding rate as a result of noradrenergic stimulation of the paraventricular nucleus (Leibowitz, 1978; Leibowitz *et al.*, 1981).

3.8. DISCUSSION

In Section 3, an attempt was made to give a survey of experimental data on the anatomy of forebrain limbic input sources to the lateral, dorsomedial, ventromedial and paraventricular hypothalamic nuclei. Although the existence of anatomical connections, at least on a light-microscopic level, appears reasonably well established, data on the functional significance of such connections are scarce and indicative of a high level of complexity. The relationship between prefrontal cortical fields and in particular the lateral hypothalamic area has only recently drawn attention in relation to control of feeding and metabolism. As a result of prefrontal cortex lesions in the rat, transient aphagia occurs, combined with impairments in the physical handling of food (an apparent motor disorder). Moreover, a change in body composition expressed in reduced body weight and an abnormal response to starvation has been described following prefrontal cortex lesions (Kolb, 1984; de Bruin *et al.*, 1983). Frontal cortex connections to the hypothalamus in the rat have also been established by means of combined anatomical-neurophysiological experiments (Kita and Oomura, 1981). More recent neurophysiological single cell recordings in awake monkeys indicate that the prefrontal cortex, at least its dorsolateral aspects, participates in a multimodal way in complex learned feeding behavior (Ono *et al.*, 1984). It may be assumed that the hippocampal formation takes part in such higher order learning and motivational functions in which the hippocampo-hypothalamic connections we described in this section may be a part. So far, however, such assumptions lie in the domain of speculation.

With respect to learning processes related to feeding, e.g. in conditioned avoidance situations, the involvement of the amygdaloid body should be included. Lesion studies on various amygdaloid nuclei may lead to loss of conditioned taste aversions or hyperphagia and increased body weight, which is the case after damage to the lateral amygdaloid nuclei (Aggleton *et al.*, 1981; Lénárd *et al.*, 1982; LePiane and Phillips, 1978). The medial amygdala has been shown to generate taste mediated drives and enhanced learning abilities after food deprivation (Schoenfeld and Hamilton, 1981). In terms of amygdaloid influence on hypothalamic neuron activity, neurophysiological studies now provide a considerable amount of data on amygdaloid monosynaptic and bisynaptic connections to dorsomedial, ventromedial and lateral hypothalamic areas. These projections either stimulate or inhibit hypothalamic electrical neuron activity (Ono and Oomura, 1975; Oomura and Ono, 1982; Renaud, 1976). Rolls (1978) provided evidence that lateral hypothalamic neurons respond to the sight of food. By electrophysiological methods he was able to demonstrate a brain circuitry from visual cortex to the lateral hypothalamic area, between which the amygdaloid body played an intermediary function. Ono *et al.* (1981) also reported on a cooperative function between LHA and amygdala in discrimination between food and non-food stimuli. More recent investigations by these authors indicate that in the above-mentioned circuitry we should include the prefrontal cortex (Ono *et al.*, 1984).

4. Intrahypothalamic Circuitry of Feeding Related Cell Groups

4.1. INTRODUCTION

So far we have described some major input pathways from higher limbic centers to the various hypothalamic nuclei that are concerned with feeding and metabolic control

mechanisms. The hypothalamus, however, cannot simply be regarded as a pass-through of limbic output to the autonomic and neuroendocrine systems, but also should be considered as an important center of integration. This implies that the hypothalamus, apart from descending, limbic higher order input, is also provided with a wealth of information from the 'milieu interieur' both via neural and humoral pathways. These ascending pathways carrying viscerosensory information have recently been reviewed by Ricardo (1983) and shall not be further dealt with in this paper.

Instead we will pay further attention to the intrinsic connectivity of the hypothalamus, since integration of incoming and outgoing information requires more than an exchange of efferent and afferent connections. Hypothalamic outgoing commands cannot be merely seen as on/off signals to autonomic or neuroendocrine cell groups. Neurophysiological and physiological experiments on the role of the hypothalamus in metabolic control clearly indicate a highly sophisticated balance between neuroendocrine activity on one hand and sympathetic vs parasympathetic autonomic activity on the other hand. This may be exemplified by the often heterogeneous effects of local hypothalamic stimulations or lesions on autonomic nerve activity and pancreatic vs adrenal hormone release (Filaretov and Filaretova, 1985; Yoshimatsu *et al.*, 1985). The setting of such an outgoing activity balance assumes the presence of intrinsic connectivity within the hypothalamus, that firstly may reflect the way the various hypothalamic cell groups participate in the various functions, and secondly that may predict how a certain hypothalamic manipulation will result in what sort of equilibrium disturbance.

As we stated before in the introductory paragraphs (Section 1.1) there are few structures in the mammalian brain so small in size and yet involved in such a wide variety of functions. The neural organization which underlies these functional complexities therefore may prove to be of equal anatomical complexity. The study of hypothalamic circuitry, as one can imagine, has long been hampered by the relatively close proximity of cell groups that often have antagonistic effects on a specific function as is e.g. the case with the ventromedial and lateral hypothalamus. This close proximity has always proved to offer major problems when applying compounds in experimental tracer studies, even when iontophoretic injection procedures were employed. Several of the short distance connections reported in previous anterograde and retrograde transport studies should therefore be interpreted with appropriate care. Most of the problems offered by the HRP and ^3H -amino acid methods, i.e. the fiber-of-passage labeling and the tracer spread problem, have been overcome by the *Phaseolus vulgaris* leuco-agglutinin tracing method. With the introduction of the PHA-L method (Gerfen and Sawchenko, 1984), for the first time we have now at our disposal a method that produces tracer injections limited to intranuclear dimensions, and that displays the labeling of axons and boutons at extremely short and extremely long distances from the injection site. In a short survey, we now will review the major interconnections at the hypothalamic level that are maintained between lateral, dorsomedial and ventromedial hypothalamic nuclei and the paraventricular hypothalamic complex. Additional remarks will be made on connections of other origin that may be relevant to the function of feeding control and metabolic regulation.

4.2. INTRAHYPOTHALAMIC CONNECTIONS OF THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS (FIG. 7)

The connections of the VMH, especially focussed on relations with the lateral hypothalamic area have received attention ever since the introduction of the hunger-satiety dual center theory for feeding control.

Injections of PHA-L made in the core of the VMH present a picture of the intrahypothalamic connections of the VMH that may be considered as representative for the entire nucleus. In these experiments one cannot speak of any substantial VMH input to the LHA. There are some terminals seen in the ventromedial parts of the LHA, in particular in the anterior aspects of the medial forebrain bundle. More impressive is a VMH projection to the dorsomedial hypothalamus, of which the medial half appears to

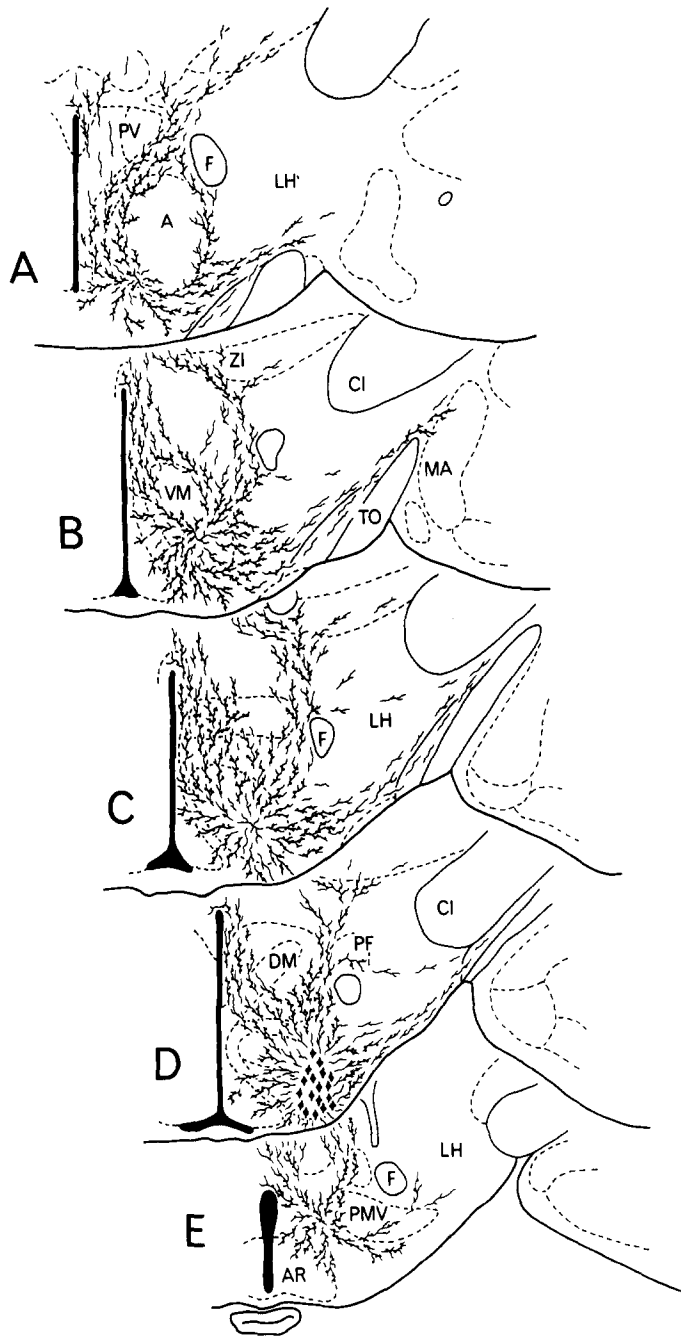


FIG. 7. PHA-L injection into the ventromedial hypothalamic nucleus (D) showing the intra-hypothalamic distribution of short-distance connections. The small dots on fiber branches represent presynaptic terminal labeling.

be more richly provided with VMH input. Other targets of VMH neurons in the hypothalamus are the periventricular column and the peripheral parts of the anterior hypothalamic nucleus. The paraventricular nucleus is conspicuously devoid of VMH input apart from some labeled passing fibers en route for other targets. Further there is some projection to the premammillary area, in particular to the dorsal premammillary nucleus. A final observation is that the VMH contains numerous efferent connections within the nucleus itself. This pattern of VMH projection is largely in line with previous reports (Luiten and Room, 1980; Kita and Oomura, 1982a) based on retrograde transport methods.

There is also some contrast, however, with findings of Kita and Oomura (1982b) who mention the retrograde labeling of cells in the VMH after injections of HRP in the lateral hypothalamic area. Such labeling, however, in part could well be the result of tracer spread from the injection locus.

We would like to stress here the general agreement there now exists concerning a VMH input to the DMH. As we will see in Section 4.4., this connection appears to be reciprocal, which throws some light on the functional significance of the DMH in the metabolic control substrate.

In conclusion we may state that the VMH cannot be regarded as a major donor of intrahypothalamic connections. Its intrahypothalamic output is largely confined to a rather diffuse periventricular zone including the medial aspects of the dorsomedial nucleus and a rich pattern of intranuclear connections. In subsequent sections, we will see that the connections of the VMH are indicative for a more outgoing function towards the autonomic nervous system, instead of being considered as a major integration center within the hypothalamus.

4.3. INTRAHYPOTHALAMIC CONNECTIONS OF THE LATERAL HYPOTHALAMIC AREA (FIG. 8)

The lateral hypothalamic area—formerly regarded as the intrahypothalamic antagonistic counterpart of the VMH in the control of feeding—is a cytoarchitectonically entirely different structure compared to the more cell-dense nuclei of the medial zone. Basically the LHA should be regarded as the nerve cell body population that accompanies the medial forebrain bundle. Yet this neuronal complex participates as an entity with its own characteristics in hypothalamic functions, that is only partially dominated by the longitudinal stream of the medial forebrain bundle.

We have studied the efferent connections of the LHA by a large number of PHA-L injections in basically all anterior–posterior levels of the LHA, and both in the core and peripheral aspects of the LHA. The pattern of intrahypothalamic connections of the various regions in the LHA is highly complex and would demand an extensive description. We will limit our description in the context of this review, however, to some more conspicuous observations. In all cases, it was obvious that, by far, most of the intrahypothalamic output of the LHA is aimed at other parts and regions of the LHA itself. This means that the LHA neurons provide a connectivity channel that serves an information exchange between the various cell groups that make up the LHA. A second major observation was that only the peripheral zones of the LHA can be regarded as a source of intrahypothalamic efferents, whereas the central core apparently is the domain of the ‘passing by’ medial forebrain bundle. In particular in the medial and ventromedial aspects of the LHA, we found most of the donors of LHA efferents to other hypothalamic structures. From these LHA areas, some projecting terminals can be observed in the VMH, both in the nuclear core and in the VMH surrounding fiber shell. This projection to the VMH, however, cannot be regarded as a quantitatively important connection. The same can be said about LHA efferents to the paraventricular nucleus, of which both parvo- and magnocellular parts receive some LHA input. Projections of the LHA to the dorsomedial hypothalamus appear to be more extensive. Essentially all anterior–posterior levels of the DMH receive this projection, but the lateral parts of this nucleus are more richly provided than the medial parts. Moreover, there is a considerable LHA input to the perifornical region, to the posterior hypothalamic nucleus and to the dorsal premammillary nucleus. Again most of these data are in agreement with data from previous HRP studies (Kita and Oomura, 1982a; Luiten and Room, 1980). Kita and Oomura (1982b), however, did not find a projection from the LHA to the DMH which is definitely present in our PHA-L material.

In conclusion, also for the LHA we may state that its connections tend to indicate this area as being more of an output station to the autonomic nervous system, rather than being a dominant integration center in the hypothalamus. As does the VMH, the LHA maintains

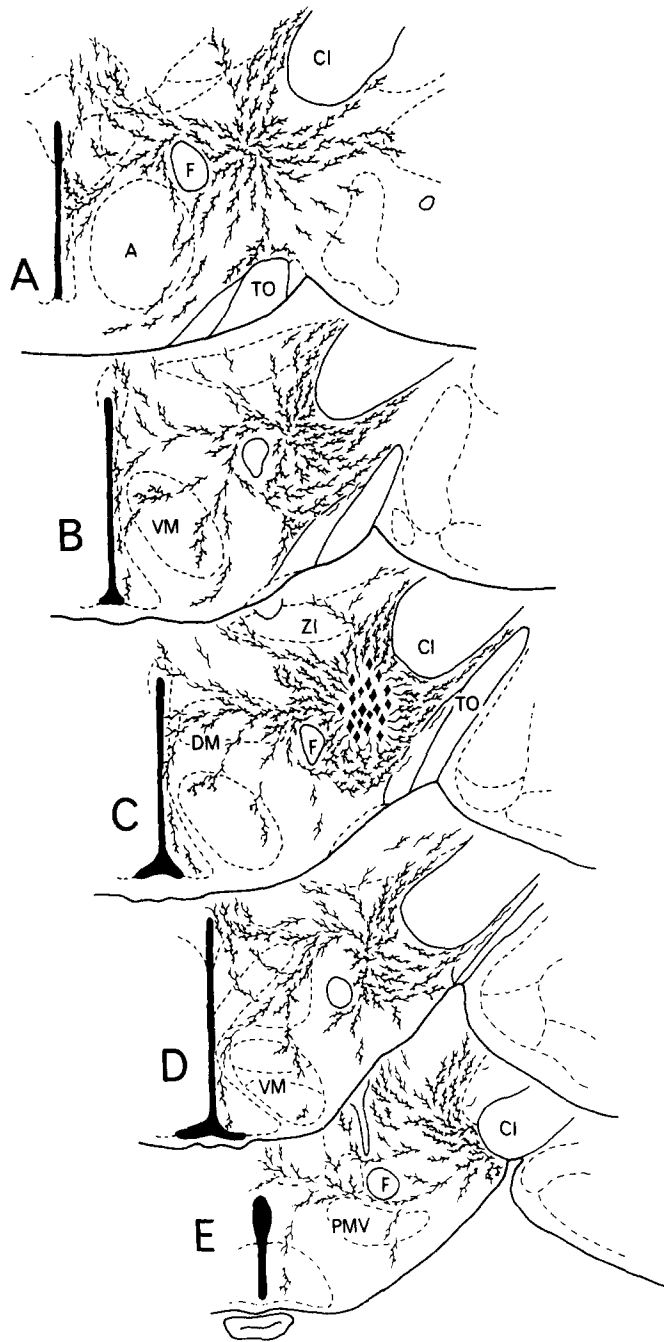


FIG. 8. Intrahypothalamic efferent connections of the lateral hypothalamic area demonstrated by a PHA-L injection in the central aspects of the LHA (C).

a reasonably well-developed connection to the DMH. Also in line with the VMH efferents, one cannot really speak of a direct link between the VMH-LHA as an antagonistic dual center for feeding control. The setting of activity balance between VMH and LHA, therefore, will either be carried out by intermediate hypothalamic structures (in which the DMH may be a candidate) or at other levels, e.g. periaqueductal grey or lower medullary reticular formation, as will be argued in the following sections. Furthermore, the LHA connections suggest a well-developed integrative circuit within the lateral hypothalamus. Connections with the paraventricular complex are present, but in a quantitative sense of moderate importance.

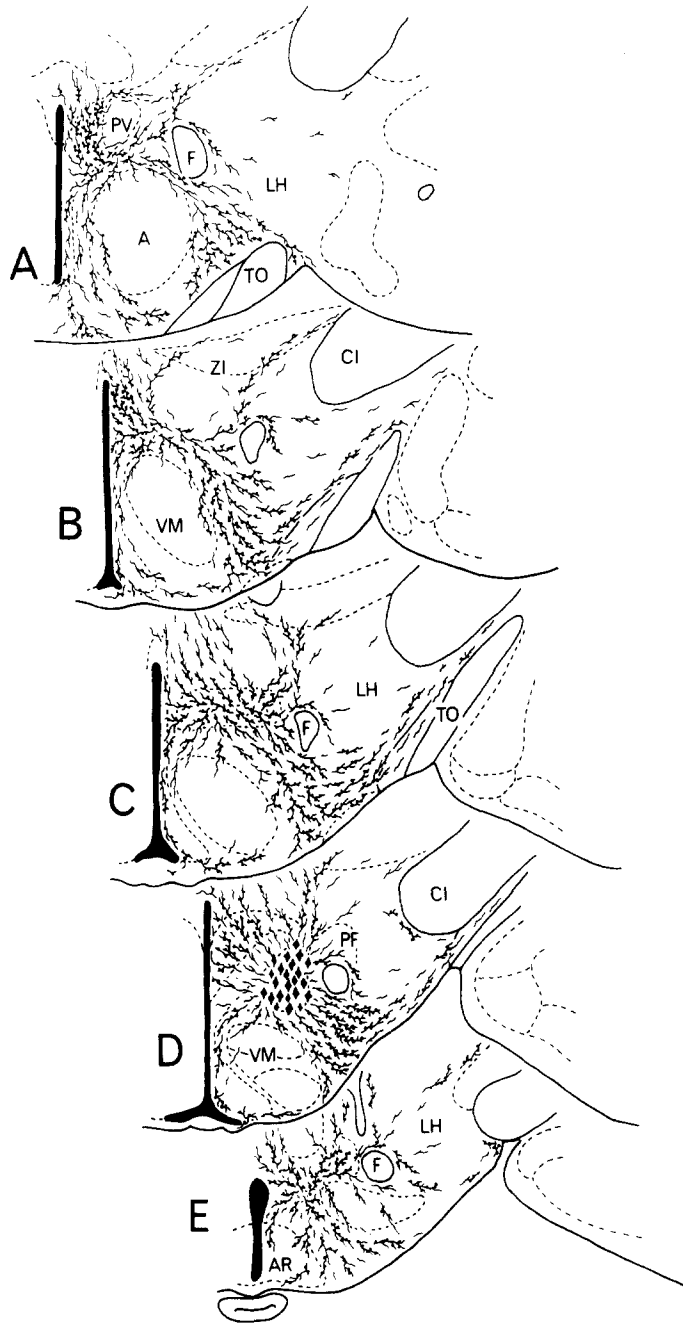


FIG. 9. Series of transverse sections of the rat hypothalamus from anterior (A) to posterior (E) in which is indicated intrahypothalamic efferent labeling of dorsomedial hypothalamic neurons injected with PHA-L (D). The relatively dense projection of the DMH to the parvocellular paraventricular nucleus in (A) has been drawn in detail in Fig. 10.

4.4. INTRAHYPOTHALAMIC CONNECTIONS OF THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS (FIG. 9)

In terms of functional relations to feeding and metabolic regulation, the DMH cannot be defined as clearly as is the case with the VMH and LHA. The function of the DMH in feeding and metabolic control has been extensively studied in weanling rats by Bernardis and coworkers (Bernardis, 1985; Bernardis and Bellinger, 1984). Following DMH lesions there are multiple changes related to feeding and metabolism that apparently are the result of autonomic nervous system and neuroendocrine disturbances. Until recently, however,

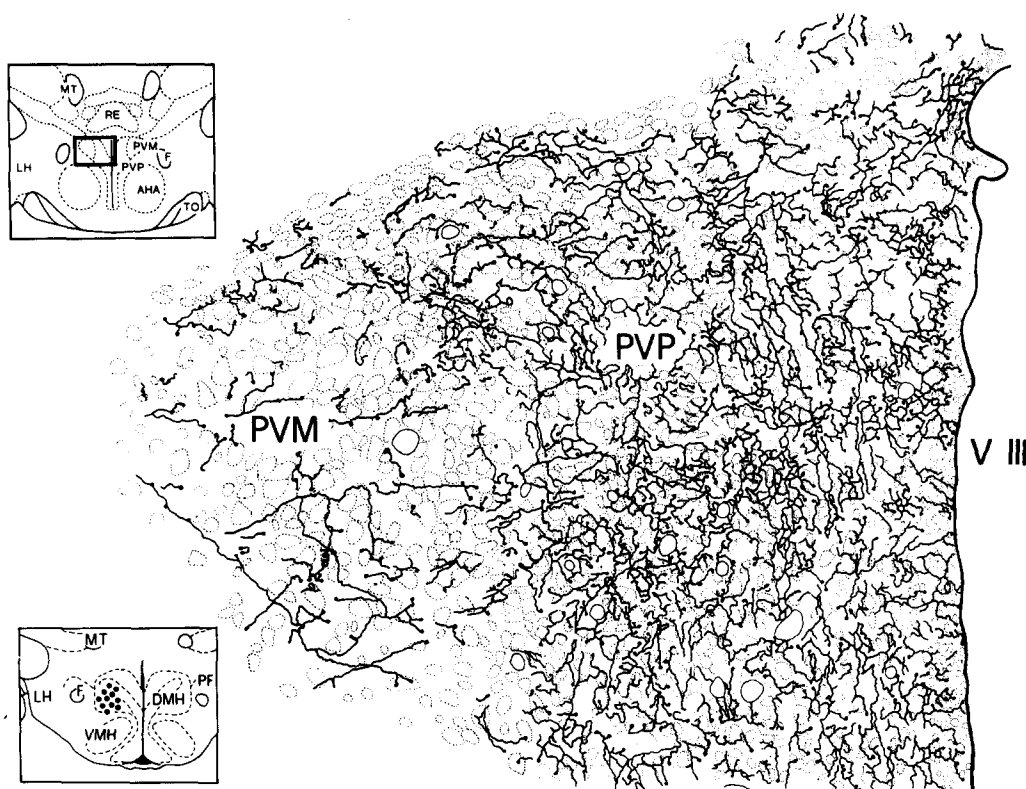


FIG. 10. Camera lucida drawing of PHA-L labeled fibers and terminal boutons in the parvocellular paraventricular nucleus (PVP) following a tracer injection in the dorsomedial hypothalamic nucleus (bottom left figure).

there was a considerable gap in our knowledge of DMH connections, which now has been compensated for as a result of the introduction of more advanced tracing methods (Ter Horst *et al.*, 1984a; Ter Horst and Luiten, 1986). These experiments reveal a pattern of DMH connections which places this nucleus in a position for a conspicuous role in feeding and metabolic regulation.

First of all, the DMH has some minor projections both to the VMH and the LHA. The projection to the VMH reaches the more peripheral parts of this nucleus. The DMH projection to the LHA is clearly limited to the medio-lateral peripheral zone of the LHA. Combined with the reciprocal VMH and LHA input to the DMH, this might indicate a bridge function for the DMH to VMH and LHA via which the latter two complexes may communicate (Fig. 11).

A more conspicuous finding was a strikingly dense projection from the DMH to the parvocellular portion of the paraventricular nucleus (Fig. 10). Accurate observation of the DMH projection to the PVN shows that the parvocellular part is the only recipient of DMH input, whereas the magnocellular cell groups are devoid of any significant terminal labeling. Similar observations were done by Sawchenko and Swanson (1983), who studied the DMH-PVN projection with autoradiographic methods. The paraventricular labeling is more or less continuous with a band of terminal labeled structures in the medial part of the anterior hypothalamic nucleus. At posterior hypothalamic levels, DMH projections to the ventral premammillary nucleus are present.

Not intrahypothalamic, but in the domain of the basal forebrain, we observed strikingly strong projections of the DMH to the subfornical organ and to the organum vasculosum of the lamina terminalis (OVLT). We would like to emphasize these projections here since we believe that these circumventricular organ projections may indicate a specific role for the DMH in the control of feedback mechanisms of bloodborne humoral factors to hypothalamic, intraventricular or basal forebrain neuronal sensors. With respect to the

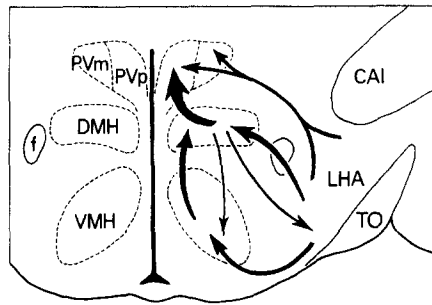


FIG. 11. Diagrammatic charting of the major intrahypothalamic connections between dorsomedial, ventromedial, lateral and paraventricular hypothalamic nuclei as described in Section 4. The thickness of the arrows are indicative for the relative intensity of the projection.

OVL projection of the DMH, it is interesting to know that we recently were able to demonstrate that the OVL specifically projects back to the DMH.

Summarizing the connections of the DMH, we may conclude that this nucleus has reciprocal relations with both VMH and LHA and as such may provide the communication between the latter two antagonistic feeding control centers (Fig. 11). Furthermore, the DMH maintains dense projections to basal forebrain circumventricular organs, in particular to the OVL, which specifically projects back to the DMH. Combined with such connections to the area postrema, as we will describe in Section 6.4, this suggests that the DMH may play a key role in the control of access to nervous tissue of humoral blood-borne compounds.

Finally, the DMH is probably one of the few hypothalamic nuclei that has a strikingly well developed input to the paraventricular nucleus. Via this PVN connection the DMH has rather direct access to the neuroendocrine system as we will further discuss in Section 5.

4.5. INTRAHYPOTHALAMIC PROJECTIONS OF THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS

There is a general consensus that the PVN, which is a functionally outstanding cell group of the hypothalamus, forms the major link between the hypothalamus and autonomic and neuroendocrine target structures. As we will describe further on, all significant efferent connections of the PVN are a reflection of the general functional characterization of this nucleus. Yet the PVN also maintains some short distance connections to hypothalamic cell groups that are worth mentioning in the context of hypothalamic function.

From the parvocellular cell groups in the PVN there are some projections to the medial and ventral peripheral zones of the LHA. Further, some efferents are aimed at the medial aspects of the VMH, whereas a fairly well-developed output is present to the DMH. A considerable projection can be observed to the perifornical column throughout the hypothalamus from anterior to posterior. As can be expected from previous retrograde transport studies, there is a rather dense projection to the periventricular layers of the third ventricle, to the arcuate nucleus and of course to the median eminence.

In conclusion we can state that all hypothalamic nuclei involved in feeding control receive a moderate PVN input and secondly that the PVN definitely should be considered as a main hypothalamic outflow station. On the other hand, the PVN is the recipient of dense projections from, in particular, the DMH. There is only a limited degree of reciprocity in this intrahypothalamic-PVN pathway in a quantitative sense. This implies that the powerful influence of the PVN on food intake and autonomic nervous activity is probably not the consequence of intrahypothalamic integration and activity processing, but rather the result of activation of the extensive PVN outgoing circuitry (Gold *et al.*, 1977; Weiss and Leibowitz, 1983).

4.6. INTRAHYPOTHALAMIC CONNECTIONS OF THE ANTERIOR HYPOTHALAMIC NUCLEUS AND VENTRAL PREMAMMILLARY NUCLEUS

There are a number of hypothalamic cell groups that so far have never been clearly demonstrated to be part of a neuronal system that subserves the regulation of feeding. Yet a large number of neuroanatomical experiments provide data that link the VMH, DMH and LHA in particular to the anterior hypothalamic nucleus (AH) and to the ventral premammillary nucleus (PMV).

In the description of intrahypothalamic circuitry and limbic input to the hypothalamus, we have seen that the AH is a recipient of projections, notably from the medial hypothalamic nuclei VMH and DMH, but also from the medial amygdaloid nuclei, ventral subiculum and ventrolateral septum.

When we review the intrahypothalamic projections of the AH as revealed by retrograde transport experiments (see Fig. 2), it is clear that the AH projects back heavily to those nuclei that are the source of AH afferents, i.e. the VMH and DMH. The AH projection to the VMH is characterized by a dense pattern of terminal fibers and boutons in the perinuclear fiber shell of the VMH (Conrad and Pfaff, 1976).

The ventral premammillary nucleus, as we have seen in Section 3.4. and Figs 5 and 6, receives a very rich and circumscribed projection from the medial amygdaloid nucleus. Lesion studies both on the medial amygdala (Bolhuis *et al.*, 1984; Luiten *et al.*, 1985a) and on the PMV (Van de Berg *et al.*, 1983) demonstrate that these interrelated nuclei participate in a brain substrate for the control of offensive and defensive behaviors. With concern for control of feeding we have reason to believe that, via the premammillary nuclei, the state of arousal in agonistic behavior is expressed in adrenal hormone release and in this way 'competes' with feeding behavior and adjusts metabolite homeostasis. Possible hypothalamic circuits that participate in such hormone release mechanisms are thought to be the projections from the PMV to the ventral half of the VMH and to the medial parvocellular portions of the PVN (Fig. 12). Both these latter nuclei, as we will see, eventually provide access to the cortex and medulla of the adrenal glands.

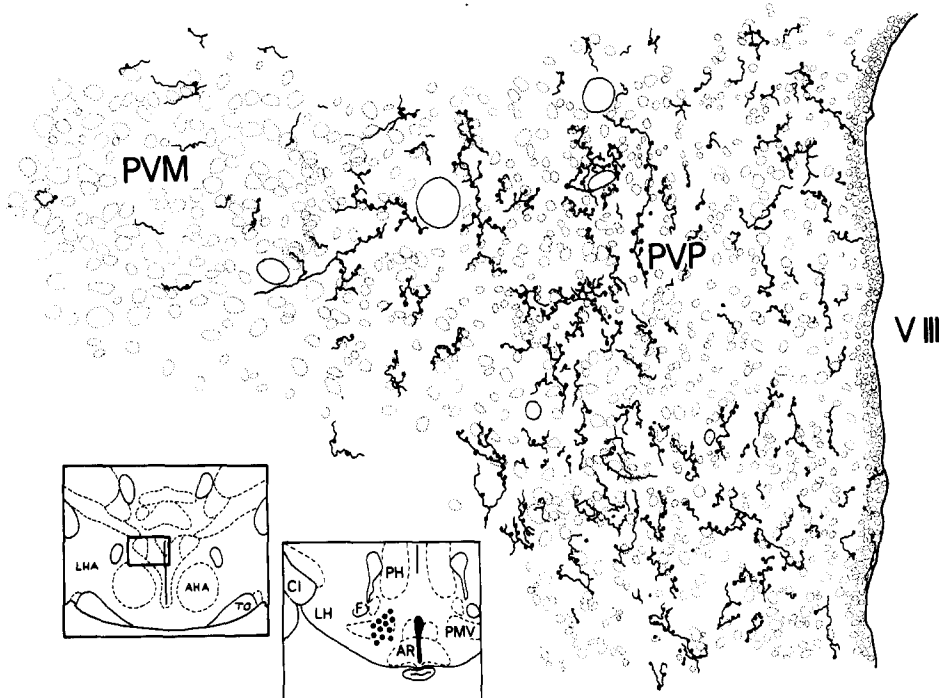


FIG. 12. Camera lucida drawing of a microscopic section with PHA-L terminal labeling in the parvocellular paraventricular nucleus as a result of a PHA-L injection in the ventral premammillary nucleus indicated by the black dots in the right bottom figure.

5. Hypothalamic Output to the Neuroendocrine System in Relation to Feeding and Metabolic Regulation

The major endocrine organ involved in feeding and homeostasis of fuel substrates, on which we have focussed our attention, is the endocrine pancreas. We should also include here the hormone-releasing cells in the gastro-intestinal tract and related organs, liver and thyroid gland. In terms of neural organization, however, we believe that the circuitry of innervation to the pancreas is basically similar for all gastro-intestinal visceral organs and is arranged according to the same concepts.

A second group of endocrine targets are the adrenal glands that have an indirect but profound influence firstly on release of pancreas hormones and metabolic homeostasis, and secondly on feeding behavior (Dallman, 1984). The adrenal hormones are considered as a class of messengers to express the state of arousal and motivation that interfere with the entire complex of functions performed by the limbic system (Brain, 1980; Henry and Stephens, 1977).

Thus in the context of the present review we will focus our attention on hypothalamic output to the endocrine system on pathways that underlie release of hormones from pancreas and adrenal glands. As we will describe in the subsequent sections, the hypothalamic connections to pancreas and adrenal medulla will most likely follow descending neural pathways to the autonomic nervous system, which seem clearly segregated from the neuroendocrine link with the adrenal cortex. This hypothalamic outflow via the neuroendocrine system is undoubtedly dominated by the PVN projections to adeno- and neurohypophysis. There is convincing anatomical and physiological evidence that the corticotropin releasing factor (CRF) containing cells in the parvocellular paraventricular nucleus, via their projections to the median eminence, constitute the major gateway in the hypothalamic expression of motivational drives and the emotional state. Via this paraventricular projection to the median eminence, releasing factors can activate the secretion of adrenocorticotrophic hormone (ACTH) from the posterior pituitary, which in turn leads to the liberation of corticosteroids from the adrenal glands (Lechan *et al.*, 1980; Merchenthaler, 1984; Merchenthaler *et al.*, 1984). A second, though minor, connection that may be included in the neuroendocrine pathway may be the dense DMH projection to the OVLT. First of all because the DMH contains a number of CRF positive cells (Merchenthaler *et al.*, 1984), secondly because the OVLT may be considered anatomically as an anterior extension of the median eminence, and thirdly because this region contains a contingent of CRF-positive fibers (Merchenthaler, 1984).

Access to the pituitary–adrenocortical axis apparently via the DMH has also been demonstrated by the impact of DMH lesions on corticosterone release in stressful situations (Filaretov and Filaretova, 1985; Grizzle *et al.*, 1974).

From the neuroanatomical data so far, it has become evident that neither the hippocampal formation, nor amygdaloid body, nor prefrontal cortex have significant direct projections to the PVN and its neuroendocrine pathways. Yet it is essential that these limbic centers, which have a strong influence on emotional states and behavior, should be able to dispose of effector channels for adrenal hormone release. In view of neuronal connectivity we have reasons to believe that the ventral premammillary nucleus (PMV) may be a candidate as a relay structure in the output from the amygdaloid body to the PVN. Such an amygdala–PVN connection is thought to consist of the amygdala projection to the PMV and the PMV projection to the parvocellular (CRF containing) part of the PVN.

So far it remains unclear, in terms of anatomical circuitry, how the hippocampal formation may exert its influence on neuroendocrine mechanisms. The prefrontal cortical regions on the other hand have well-developed and well-studied connections—mainly via the medial prefrontal region (Figs 3 and 4)—to the lateral hypothalamic area. The connections of the LHA to both parvo- and magnocellular parts of the PVN provide an anatomical basis for prefrontal control of the pituitary–adrenal axis.

Apart from the prevailing PVN pathways to the pituitary, there are a large number of additional hypothalamic neuroendocrine connections that, however, cannot be, or are only

indirectly, related to hypothalamic control functions in feeding and metabolism. We did recently find direct projections from the DMH to the neural lobe of the pituitary, but it is most likely that this concerns an additional vasopressinergic projection to the posterior pituitary (Caffé and Van Leeuwen, 1983). Furthermore, there is increasing immunocytochemical evidence, that various hypothalamic nuclei notably in the medial column of hypothalamus such as VMH, DMH, PVN and arcuate nucleus, contain a large variety of peptides. Via hypothalamo-infundibular pathways these substances may reach the ME and influence the adenohypophysis (Krieger 1983). Apart from the above described CRF pathways, it remains unclear so far how compounds like growth hormone, thyroid hormone and related releasing factors, also compounds like β -endorphin, cholecystikinin and somatostatin participate in the highly complex physiological processes that control metabolism; in the trophic and adaptive changes which continuously occur, e.g. during daily rhythms; and also during development and aging.

6. Hypothalamic Projections to the Autonomic Nervous System

6.1. INTRODUCTION

The neuroendocrine pathways we described in the previous section may constitute the most direct, yet relatively slow way for the CNS to express its output in hormonal responses. A second way by which the limbic system, via its hypothalamic output stations, reaches endocrine target organs is by way of the autonomic nervous system. We will now review our present knowledge on the neural pathways from the various hypothalamic feeding related nuclei to the endocrine target cells in the gastro-intestinal tract and adrenal glands. As a model target structure in the gastro-intestinal channel and derived organs, we have limited our direct interest to the endocrine pancreas. We believe that the pathways we will describe underlie the general hypothalamic outflow to the mechanism of digestion and related hormonal supply that eventually regulate fuel substrate homeostasis.

6.2. AUTONOMIC CONNECTIONS OF THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS

6.2.1. *Descending projections of the VMH*

The projections that originate from the ventromedial hypothalamic nucleus were studied by us and several others with a great variety of retrograde and anterograde tract-tracing methods (Krieger *et al.*, 1976; Sofroniew and Schrell, 1980, 1981; Swanson and Kuypers, 1980; Ter Horst *et al.*, 1984).

The pattern of descending projections of the VMH that can be obtained from the work of Krieger *et al.* (1979), Saper *et al.* (1973) and our own experiments on autoradiographic tracing of tritiated amino acids will be demonstrated here by an example of ^3H -leucine injection in the ventrolateral portions of the VMH (Figs 13 and 14). Such small injections can be achieved by means of iontophoretic delivery methods and yield the following results. From the injection locus two fiber bundles can be followed in a descending direction. The main bundle courses in a periventricular position and connects directly to the mesencephalic continuation of the periventricular region: the periaqueductal gray (PAG) (or substantia grisea centralis, SGC). Here a substantial increase in radioactive labeling can be observed in the dorsal and lateral quadrants of the SGC (Fig. 14). From both our autoradiographic and more recent PHA-L tracing experiments, there is no doubt that the VMH cells proper do not project far beyond the level of the mesencephalic periaqueductal gray. We may conclude that the periaqueductal gray is the major descending projection target of the VMH. Additional projections of the VMH can be observed at the same mesencephalic level. Several fibers fan out from the SGC and reach a diffuse area of the dorsal mesencephalic tegmentum. The same mesencephalic structures are the projection field of a second VMH efferent pathway, which from the VMH takes

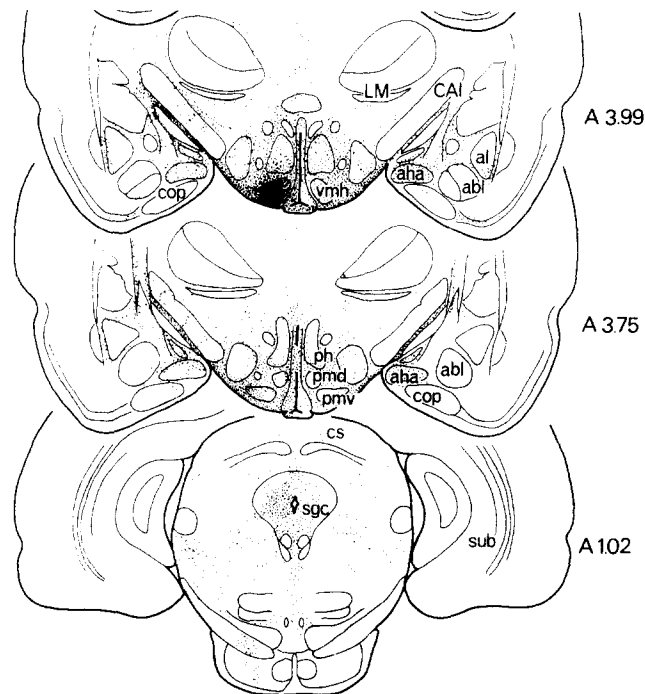


FIG. 13. Autoradiographic charting of descending projections from the ventromedial hypothalamic nucleus following a ^3H -leucine injection into the VMH. Note the relatively strong anterograde labeling at level A 1.02 in the periaqueductal gray (or substantia grisea centralis) (SGC), which indicates the major descending projection target of the VMH.

a descending course in the floor of the hypothalamus ventral to the medial forebrain bundle. These fibers run caudally and appear to end predominantly in the peripeduncular nucleus and adjacent mesencephalic tegmentum.

Again we should emphasize here that in our anterograde tracing experiments, in which the tracer injection was confined to the VMH, only limited labeling occurred of fibers descending to lower brainstem levels. Only a minor quantity of descending connections was observed to the locus coeruleus and medullary reticular formation.

The descending VMH efferent connections obtained with anterograde tracing experiments were also studied with retrograde transport methods (Grofova *et al.*, 1978; Morrell *et al.*, 1981; Ter Horst *et al.*, 1984b). Indeed, when small injections of HRP were placed in the midlateral segments of the SGC (Fig. 15), exceedingly rich retrograde labeling of cell bodies occurred in the VMH suggesting that a very large proportion of the VMH cells participate in this SGC projection. In addition to the VMH labeling we further mention here the retrograde labeling of cells in the lateral hypothalamic area and also DMH, although far less numerous than in the VMH. A few labeled somata, furthermore, appeared in the lateral parvocellular parts of the PVN.

6.2.2. Descending projections from the periaqueductal gray to the medullary reticular formation

The relatively dense and rather circumscribed VMH–SGC projection that appears to dominate the descending VMH output pathways, made us study the output connections of this particular section of the periaqueductal gray towards the lower brainstem. In this descending circuitry we hypothesized a relay function for the SGC in an output pathway from the VMH to the autonomic centers in brainstem and spinal cord.

The efferents of the SGC were studied again, both with anterograde (^3H -Leu and PHA-L) and retrograde transport methods. The anterograde tracers were injected specifically in those areas of the periaqueductal gray that are the recipients of a dense input

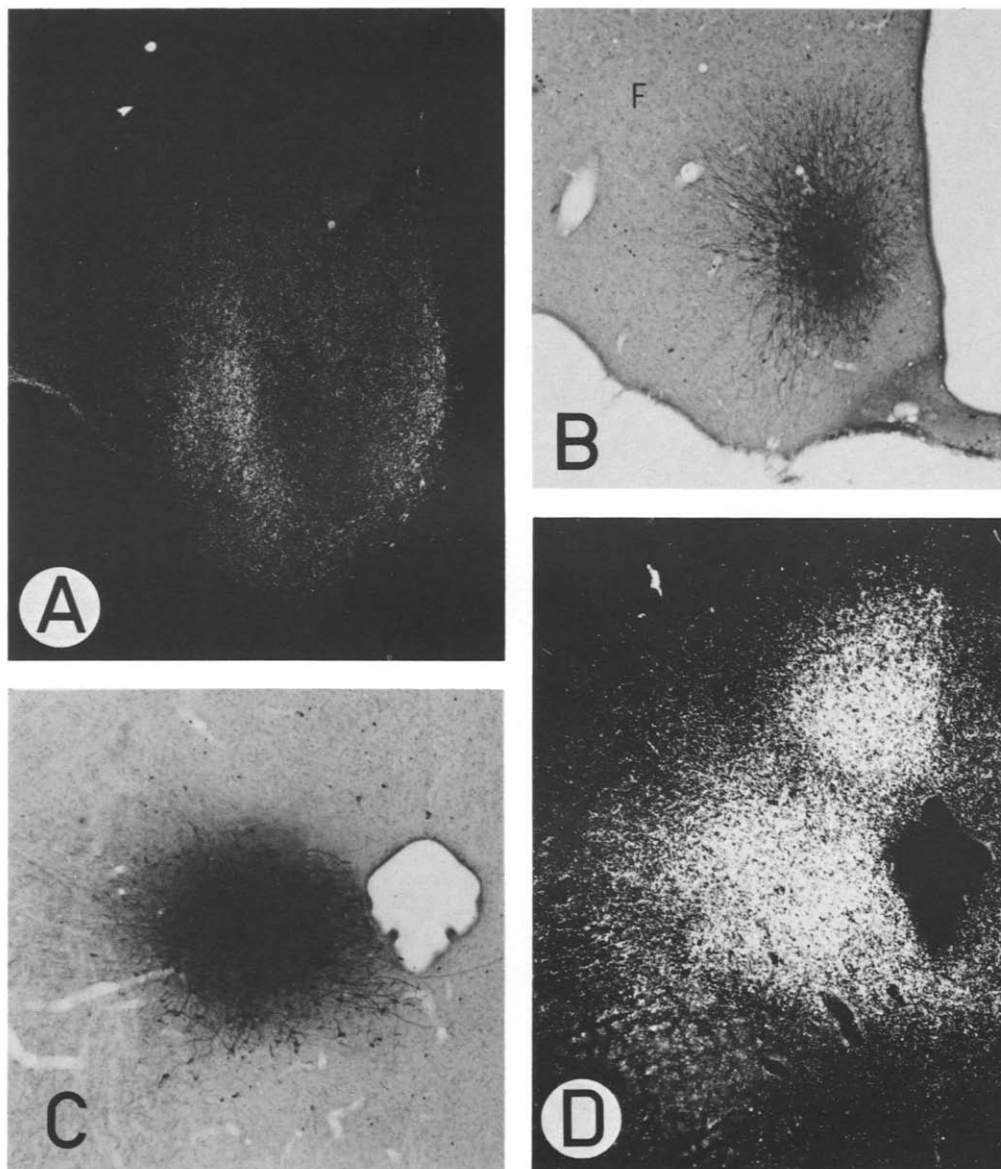


FIG. 14. (A) Photomicrograph of terminal labeling in the perinuclear shell of the VMH after a ^3H -leucine injection in the medial amygdaloid nucleus. Darkfield illumination. (B) PHA-L injection in the VMH. (C) PHA-L injection in the midbrain periaqueductal gray, which is the recipient of the descending VMH projection. Compare with (D). (D) Darkfield photomicrograph of autoradiographic terminal labeling in the periaqueductal gray after a ^3H -leu injection in the VMH. Compare with Fig. 13-A1.02.

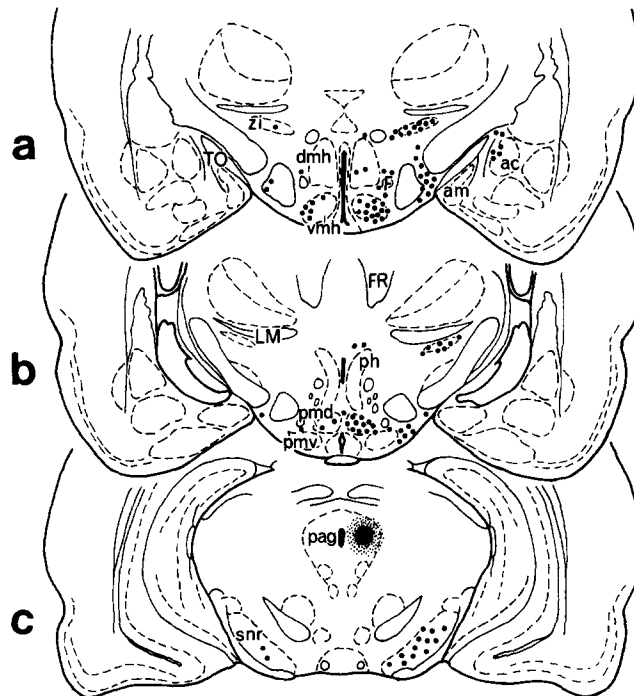


FIG. 15. Diagrammatic drawing of retrogradely labeled cell bodies at hypothalamic levels following an HRP injection in the mesencephalic periaqueductal gray.

from the VMH. The descending efferents from this SGC area, both in ^3H -Leu and PHA-L experiments, are organized in the following pattern, as can be seen in Fig. 16. From the injection site in the midlateral section of the SGC, a large contingent of labeled fibers emanates from the periaqueductal gray in a ventrolateral direction and courses caudally in a ventral position. These fibers initially run medial to the lateral lemniscus, but at medullary levels they are found parallel to the raphe magnus column. In the descending course of these SGC pathways, various projections can be observed to the locus coeruleus and to the subdivisions of the reticular formation of the meten- and myelencephalon. At lower medullary levels, a number of projections occurred that we consider as essential for descending autonomic circuitry.

One major termination field can be discerned in the ventromedial reticular formation immediately dorsal to the inferior olive complex (Fig. 16c). A second dominant site of presynaptic endings occurs in the reticular area surrounding the nucleus ambiguus (see also Mantyh, 1983) (Fig. 16c,d). This latter area is defined as the parvocellular reticular nucleus (RPC), posterior extension of which is called the dorsal medullary nucleus (MDD) (Kalia and Fuxe, 1985). At the same level, SGC projections can be detected to the ambiguus (AMB) and dorsal vagal motor nuclei (DMV), which are the seat of the preganglionic neurons of the parasympathetic division of the autonomic nervous system (ANS) (Luiten *et al.*, 1984; Weaver, 1980). In particular, the SGC projection to the AMB appears to be well developed. Beyond the level of the lower medulla, a number of fibers can be followed within the lateral funiculus of the spinal cord that appear to end in the intermediolateral zone of the upper thoracic segments. These spinal projections of the SGC, however, are sparse, which is in agreement with the findings of Holstege and Kuypers (1982) and Leong *et al.* (1984), although Mantyh and Peschansky (1982) describe more abundant spinal projections from the SGC.

6.2.3. Spinal projections from the ventromedial reticular formation

Lectin injections in the ventromedial reticular formation, in the area dorsal to the inferior olive (IO) and medial to the paragigantocellular nucleus (PGi), give rise to

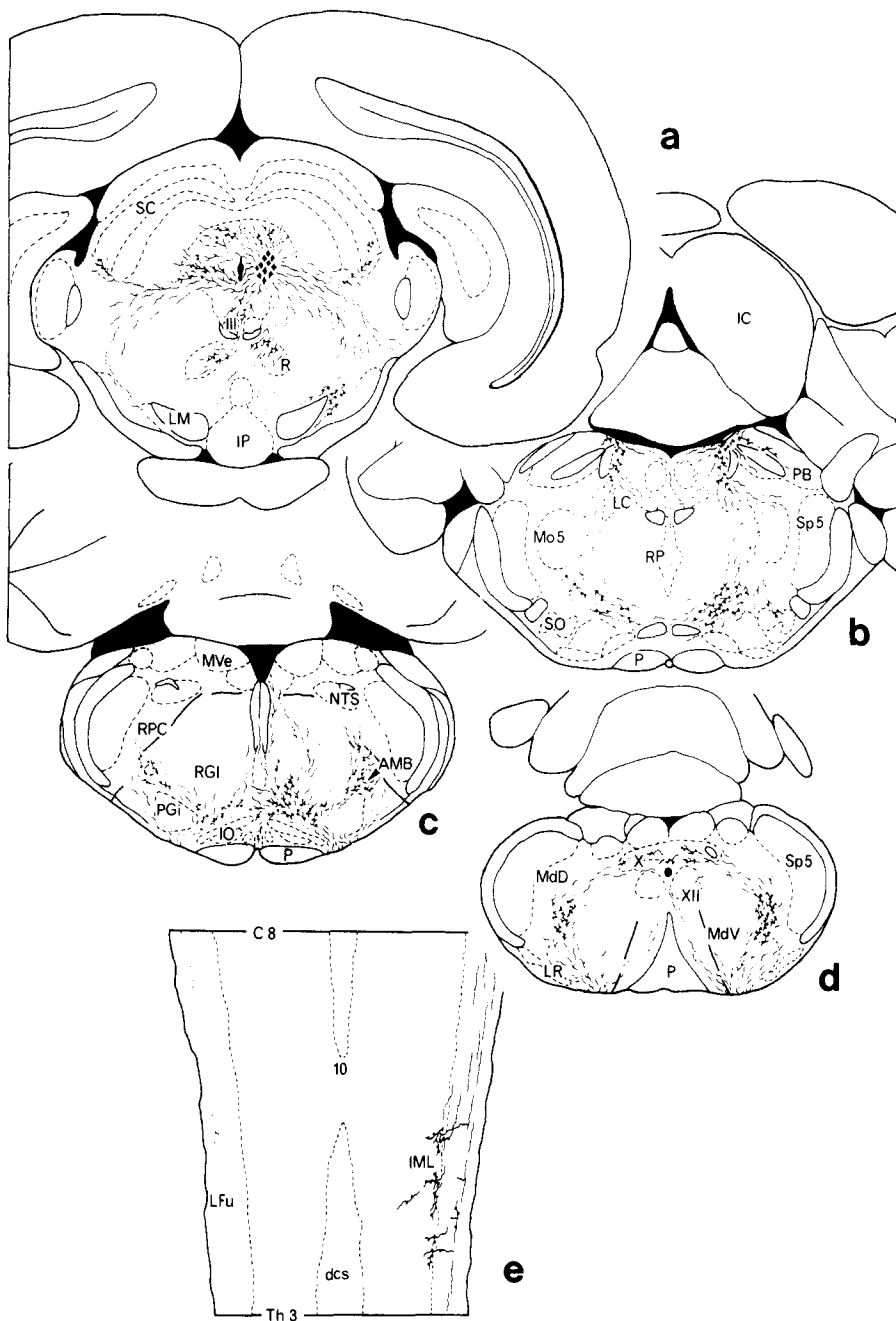


FIG. 16. Anterograde labeling of descending axons and synaptic terminals after a PHA-L injection in the central parts of the midbrain periaqueductal gray (a). In (c) and (d) projections to the lower medullary reticular formation are indicated, whereas in (e) a longitudinal section of the thoracolumbar cord is given.

abundant spinal projections, both at the cervical and thoracic cord levels (Fig. 17). The efferent fibers travel in a posterior direction between IO and the lateral reticular nucleus (LRt). A second descending bundle runs lateral between LRt and spinal trigeminal nucleus (SP5). The labeled fibers could be traced in the lateral funiculus (LFu) as far as the lumbar cord level. Projections in the cervical cord are found onto ventral horn cell groups, predominantly situated adjacent to the medial part of the ventral funiculus (VFu), whereas they are absent in the C₃–C₄ group that innervates the endocrine pancreas (see Section 7). At the thoracic level the efferent fibers branch again and give rise to collaterals that are richly provided with presynaptic boutons and that run parallel to the LFu in the

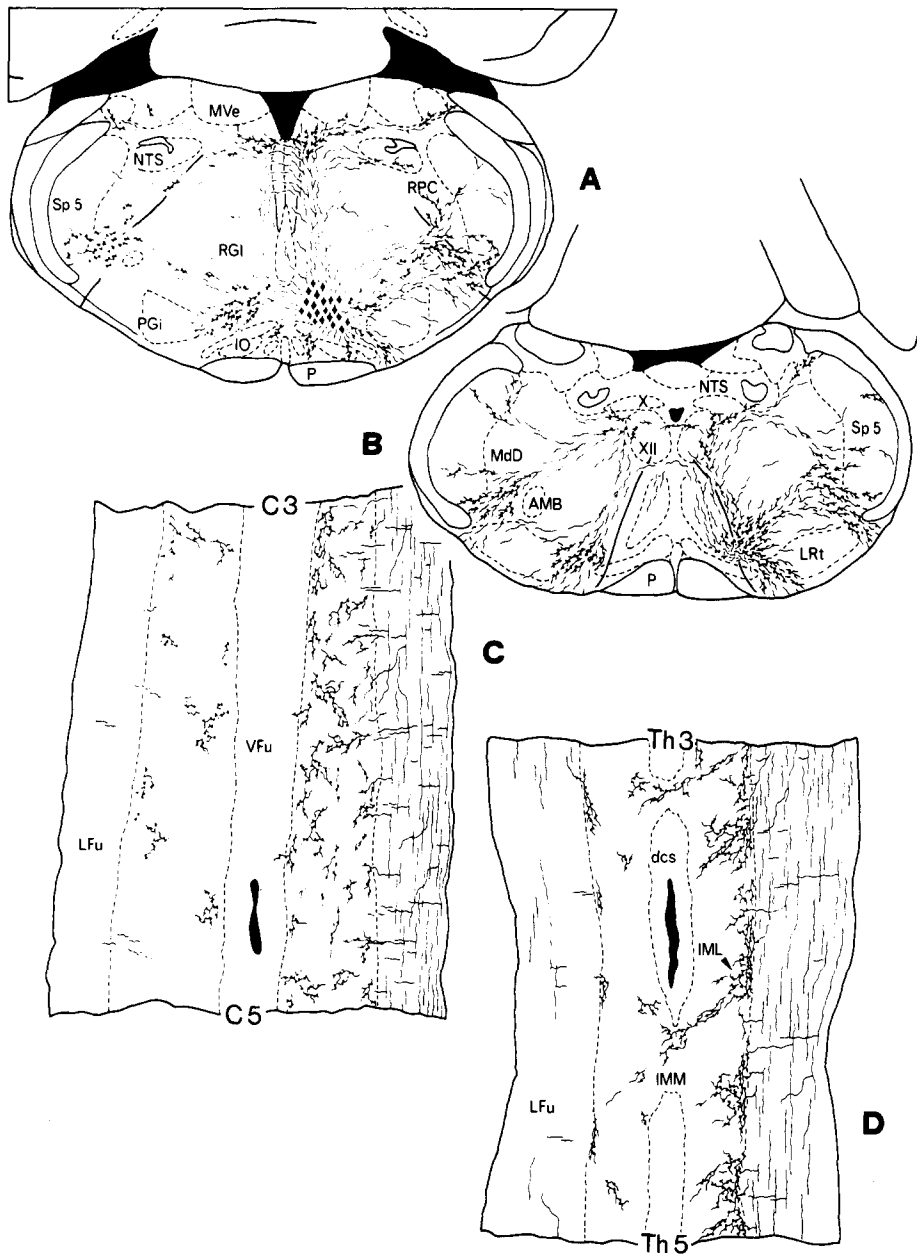


FIG. 17. Anterograde tracing of spinal projections from the ventromedial reticular formation of the lower medulla oblongata after local PHA-L injections (A). Note the particularly heavy terminal labeling in the intermediolateral column of thoraco-lumbar cord. (A) and (B) are transverse, (C) and (D) are horizontal sections.

intermediolateral cell column (IML). In addition to this IML projection, terminal labeling is also found in the intermediomedial area (IMM). Both IML and IMM contain preganglionic sympathetic cells that innervate the endocrine pancreas.

These anatomical results show that the ventromedial reticular formation fulfills a predominantly sympathetic function in the autonomic control of the endocrine pancreas, which is also emphasized by the absence of projections to the parasympathetic cell groups in the dorsal motor vagus and ambiguous nuclei.

6.2.4. Spinal projections from the parvocellular reticular formation

The significance of the RPC and MDD in the distribution of descending autonomic outflow to both sympathetic and parasympathetic cell populations becomes apparent both

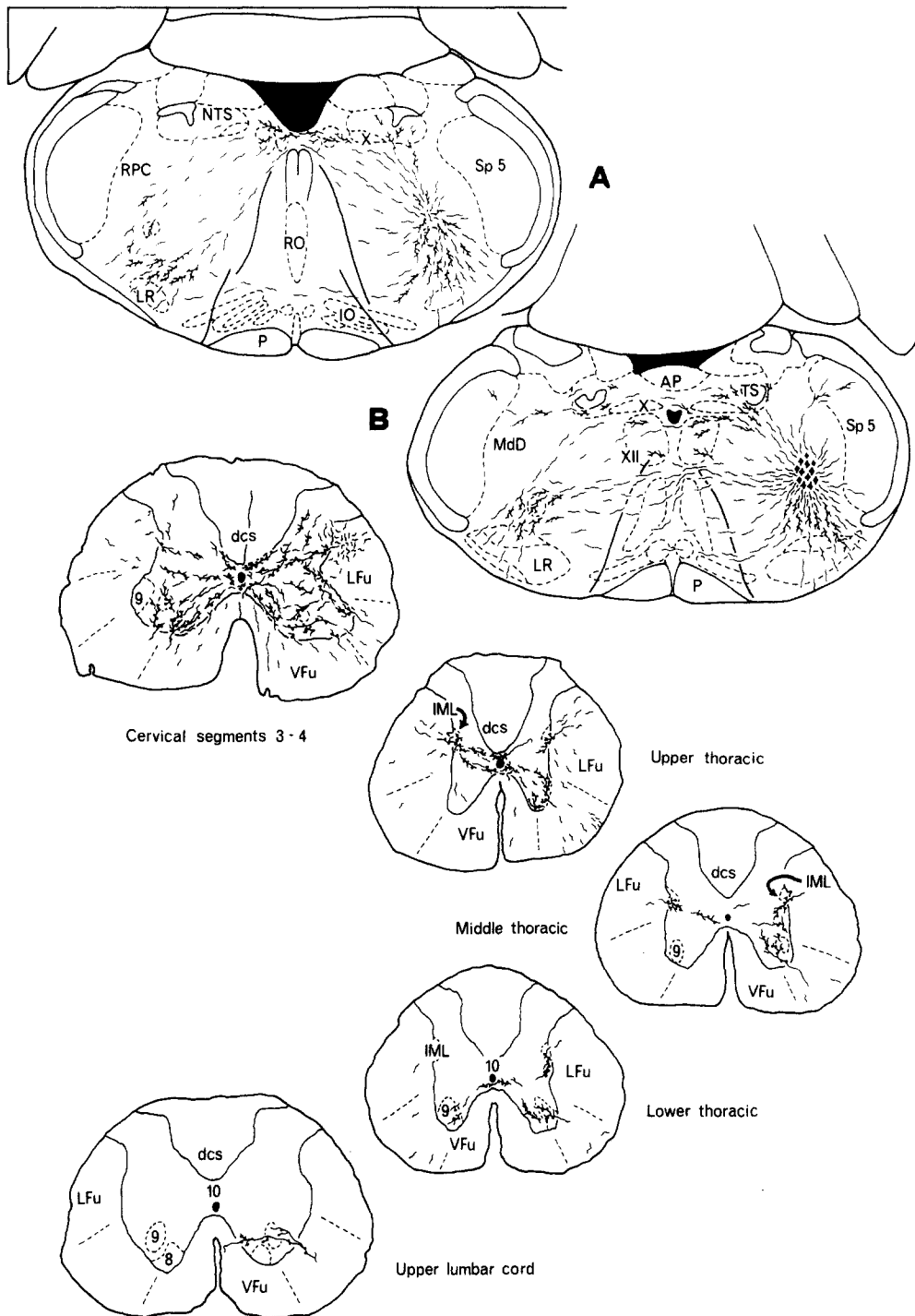


FIG. 18. Series of transverse sections of lower medulla and various levels of the spinal cord, with the descending efferent connections of the parvocellular and dorsal medullary reticular nucleus (resp. RPC and MDD) as demonstrated with *Phaseolus vulgaris* lectin tracing.

from its connectivity and from the effects of neurophysiological stimulation that we will describe in Section 8. The RPC and MDD provide the following set of efferent connections (Fig. 18). It is striking that the RPC and MDD do not maintain any significant projections to the parasympathetic cell groups in DMV and AMB, but have a strong output to the lateral part of the ventral reticular nucleus of the medulla, situated on top of the lateral reticular nucleus (LR). Moreover, the RPC/MDD area projects heavily on a number of

spinal structures via the lateral funiculus. Most prominent are the projections to the intermediolateral (IML) and intermediomedial (or area 10 of Rexed) (Molander *et al.*, 1984) columns of the thoracic cord. This latter RPC-IML projection may be interpreted as a second link in a descending circuit to the sympathetic cell groups of the ANS. The projection of the RPC to the ventral medullary reticular nucleus (MDV) gains importance in autonomic connections, since it contains the noradrenergic and adrenergic cell groups A₁/C₁ (Ross *et al.*, 1984). We were able to demonstrate a descending pathway from the MDV to a longitudinal pool of large efferent motor neurons in the cervical cord segments 3–4. As we will describe in Section 7, this pool of motor cells most likely constitutes a second group of preganglionic parasympathetic neurons that innervate the endocrine pancreas (Luiten *et al.*, 1984).

In conclusion, we have described here a seemingly complicated descending circuit that originates from the ventromedial hypothalamic nucleus, which projects via a relay in the periaqueductal gray to the parvocellular reticular areas of the medulla. Both areas, but in particular the ventromedial reticular formation, were found to connect with the sympathetic regions of the thoracic and lumbar spinal cord. This preponderantly sympathetic outflow channel from the VMH was not only studied by anterograde tracing experiments, but could be partly confirmed by HRP tracing as well (Ter Horst *et al.*, 1984b).

6.3. DESCENDING AUTONOMIC CONNECTIONS OF THE LATERAL HYPOTHALAMIC AREA

Compared with the rather complex descending autonomic circuit of the VMH, the descending pathways that originate in the lateral hypothalamic area appear to be less complicated. The descending LHA connections differ from the VMH pathways in having direct projections to the parasympathetic nuclei of the lower brainstem. An overall presentation of descending LHA projections is given in Fig. 19, in which PHA-L was injected in a large part of the LHA. Two major bundles of descending efferents can be discerned, a more dorsal periventricular bundle and a more ventral bundle that caudalwards takes a ventromedial position. From these two bundles of fibers, small fascicles can be seen aiming at terminal structures along the descending course between hypothalamus and medulla. Major projection fields can be observed in the ventrolateral quadrant of the caudal aspects of the periaqueductal gray (PAG) and in the adjacent dorsal tegmentum. More caudal, a considerable projection is present in the tegmental pedunculo-pontine nucleus and in the parabrachial nucleus. At the lower medulla levels, a rather diffuse pattern of termination occurs over the longitudinal extent of the ambiguous nucleus, the ventrolateral reticular formation that surrounds the AMB, which for a part is made up by the RPC, and in the nucleus of the solitary tract. Furthermore, a fairly heavy projection to the dorsal motor vagus (DMV or X) motor pool is apparent in our material. A small number of fibers continue their caudal course into the spinal cord and appear to give off limited numbers of terminations in the intermediate column of the cervico-thoracic segments. These projections, however, do not appear to be linked directly to sympathetic cell groups. Only part of these projections have been described in anterograde transport studies by Saper *et al.* (1979), but more recent autoradiographic studies by Hosoya (1985) and Hosoya and Matsushita (1981) basically provide a similar medulla projection pattern as we described here in PHA-L material.

Most of the LHA projections mentioned above were also demonstrated by means of retrograde transport techniques (Hancock, 1976; Haring and Davis, 1983; Leong *et al.*, 1984; Ono *et al.*, 1978; Saper *et al.*, 1976; Ter Horst *et al.*, 1984b). The direct LHA projections to the parasympathetic motor nuclei of the medulla could well be demonstrated after small HRP injections confined to the dorsal motor vagus nucleus which result in retrograde cell labeling in the more peripheral zones of the LHA (Fig. 20). A comparable set of LHA labeling can be obtained by HRP injections in the AMB and RPC (Ter Horst *et al.*, 1984b). The retrograde labeling in the periaqueductal gray after HRP injections in

dorsal motor vagus and ambiguous nuclei, combined with LHA projections to the PAG indicates that the LHA participates in indirect descending pathways as well.

We may summarize the LHA-autonomic connections in the following way. The outgoing pathways from the LHA consist both of direct and indirect circuits that are predominantly aimed at the parasympathetic cell groups of the lower medulla. The direct circuit is made up of rather diffuse LHA projections to DMV and AMB. The indirect circuit includes a relay in the more ventral regions of the periaqueductal gray and the parvocellular reticular formation which provides entry to both sympathetic and parasympathetic autonomic cell groups. This indirect circuit partly shares the neural pathways for the VMH output.

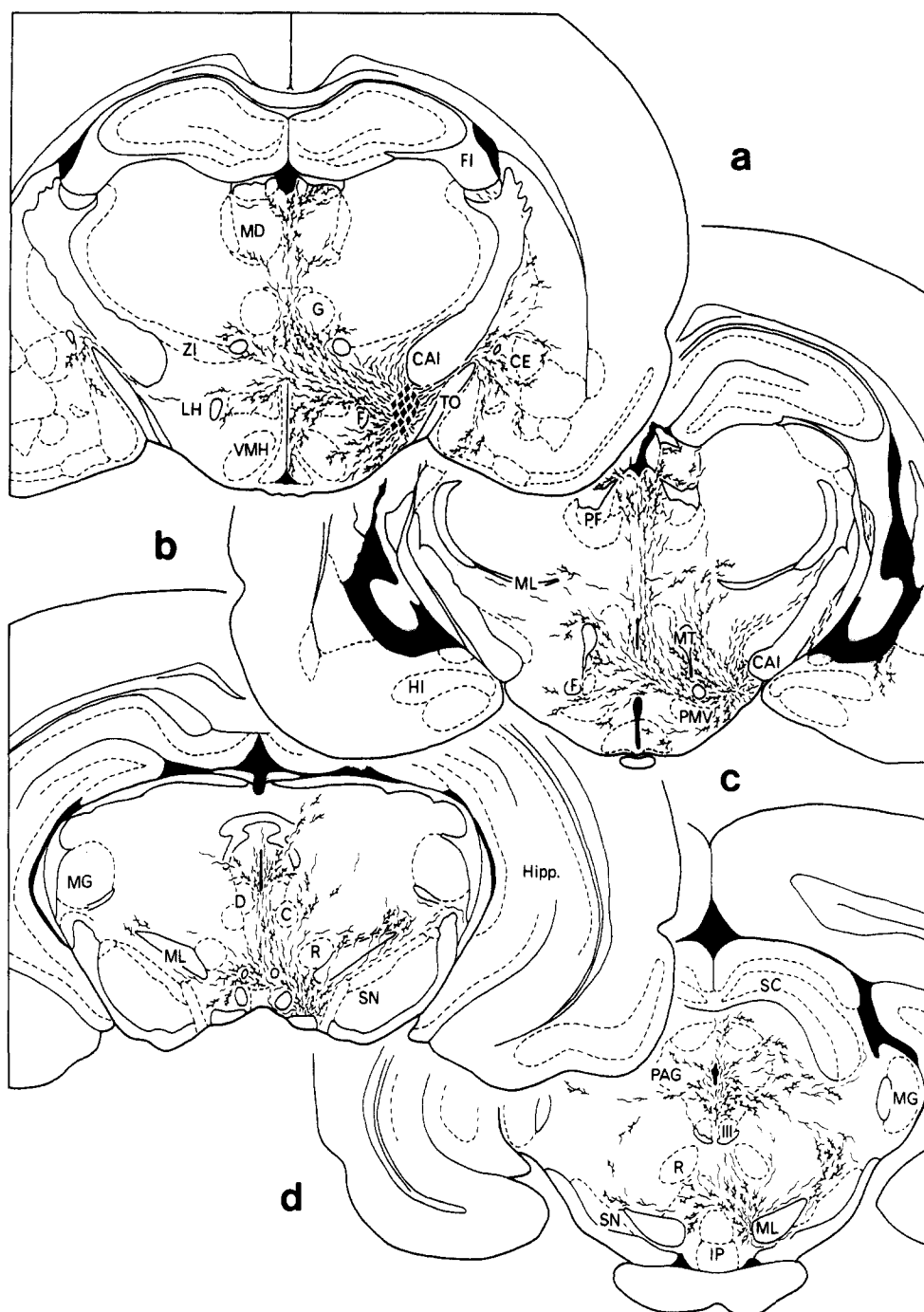


Fig. 19.

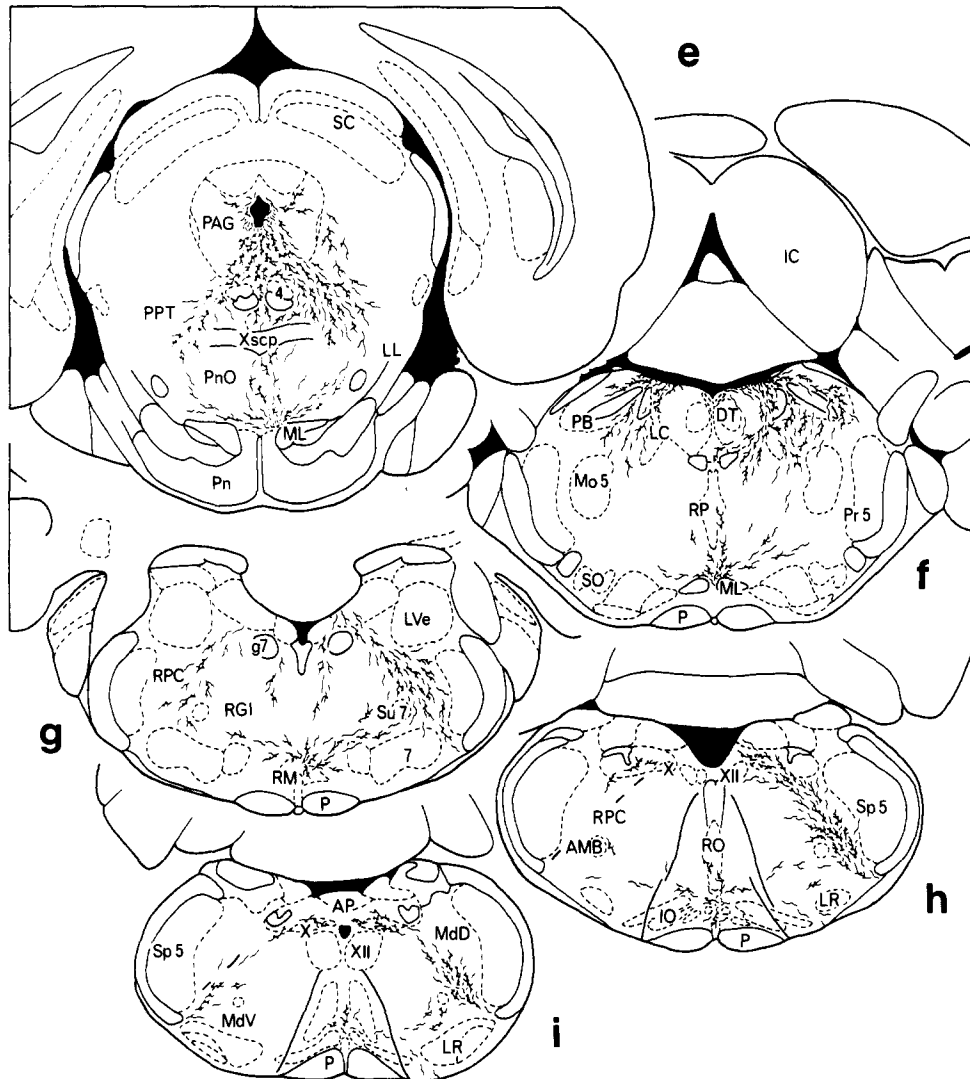


FIG. 19. Transverse sections through the brain from the level of the hypothalamus (a) to the lower medulla oblongata (i) illustrating anterogradely PHA-L labeled projections that originate from the lateral hypothalamic area. The black cell group in section (a) indicates the site of injection.

6.4. DESCENDING AUTONOMIC CONNECTIONS OF THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS

In Section 4.4. we concluded that the dorsomedial hypothalamic nucleus fulfills its major role in relaying intrahypothalamic communication between VMH, LHA and PVN. At the same time, however, the DMH itself maintains its own output pathway, not only in the neuroendocrine system but to the autonomic nervous system as well. When the efferent connectivity of the DMH is compared with the projection pattern of the LHA, it is an obvious conclusion that both hypothalamic structures maintain rather similar outgoing pathways, with the only distinction that the descending DMH projection is somewhat less abundant. In PHA-L labeled material, two fiber bundles emanate from the DMH, one taking a dorsal periventricular and periaqueductal position, the other running ventro-medial close to the midline (Fig. 21). From anterior to posterior, terminating labeled structures can be discerned in the midlateral periaqueductal gray (CG in Fig. 21E), in the ventral tegmental area, locus coeruleus and parabrachial nuclei and in the AMB, RPC and DMV (X in Fig. 21H, I). In addition, we found a DMH projection to the area postrema. In our material the DMH does not give rise to any fibers aimed at spinal structures.

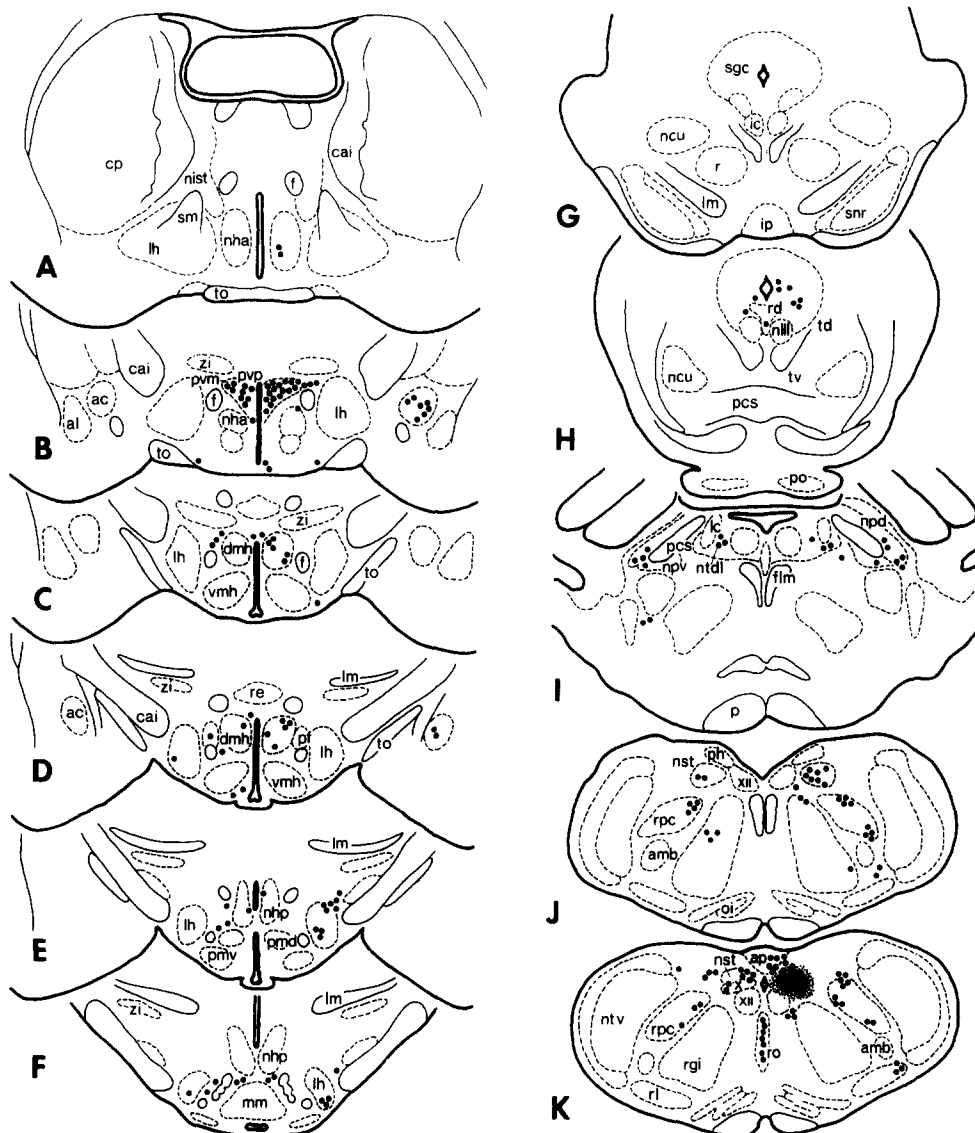


FIG. 20. Retrogradely labeled nerve cell bodies at various diencephalic and mesencephalic levels as a result of HRP injections in the dorsal motor vagus nucleus (X in section K).

Retrograde labeling with HRP, that support these anterograde tracing experiments, can be seen in Figs 15 and 20 with HRP deposits in periaqueductal gray and DMV. As is the case with LHA, we may draw the same conclusion that the DMH is predominantly aimed at parasympathetic cell groups that may be reached by direct and partly indirect connections. Further support for this conclusion is also given by the effects of DMH lesioning on vagal and splanchnic pancreatic nerve activity as studied by Yoshimatsu *et al.* (1984). As a result of DMH damage a considerable increase in parasympathetic, but only a minor change in sympathetic activity can be established.

6.5. DESCENDING AUTONOMIC CONNECTIONS OF THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS

It is now a well-established fact that, in the context of feeding and metabolic regulation, the paraventricular nucleus is the most effective site from where feeding behavior can be elicited. Secondly, the PVN is the main link between hypothalamus and pituitary in the neuroendocrine system and thirdly, it gives rise to a unique network of connections to all

autonomic cell groups in brainstem and spinal cord (Buijs, 1978; Leibowitz, 1978; Leibowitz *et al.*, 1981; Luiten *et al.*, 1985b; Ono *et al.*, 1978; Saper *et al.*, 1976; Swanson and Hartman, 1980; Swanson and Kuypers, 1980; Swanson and Sawchenko, 1983). The entire course of efferent fibers from the paraventricular nucleus including their terminations can be illustrated by application of PHA-L methods. After injection of this tracer in the parvocellular portions of the PVN, which is the subdivision of the PVN that gives rise to the autonomic descending pathways (Swanson and Kuypers, 1980), two main-streams of descending fibers can be discriminated (Fig. 22). One fiber tract (bundle 1 in Fig. 22) maintains a periventricular position. A second bundle (2) runs lateral over the substantia nigra and eventually finds a position in the floor of the brainstem. These descending pathways provide synaptic endings in the periaqueductal gray (PAG) and Edinger-Westphal nucleus, ventral mesencephalic tegmentum, pedunculo-pontine teg-

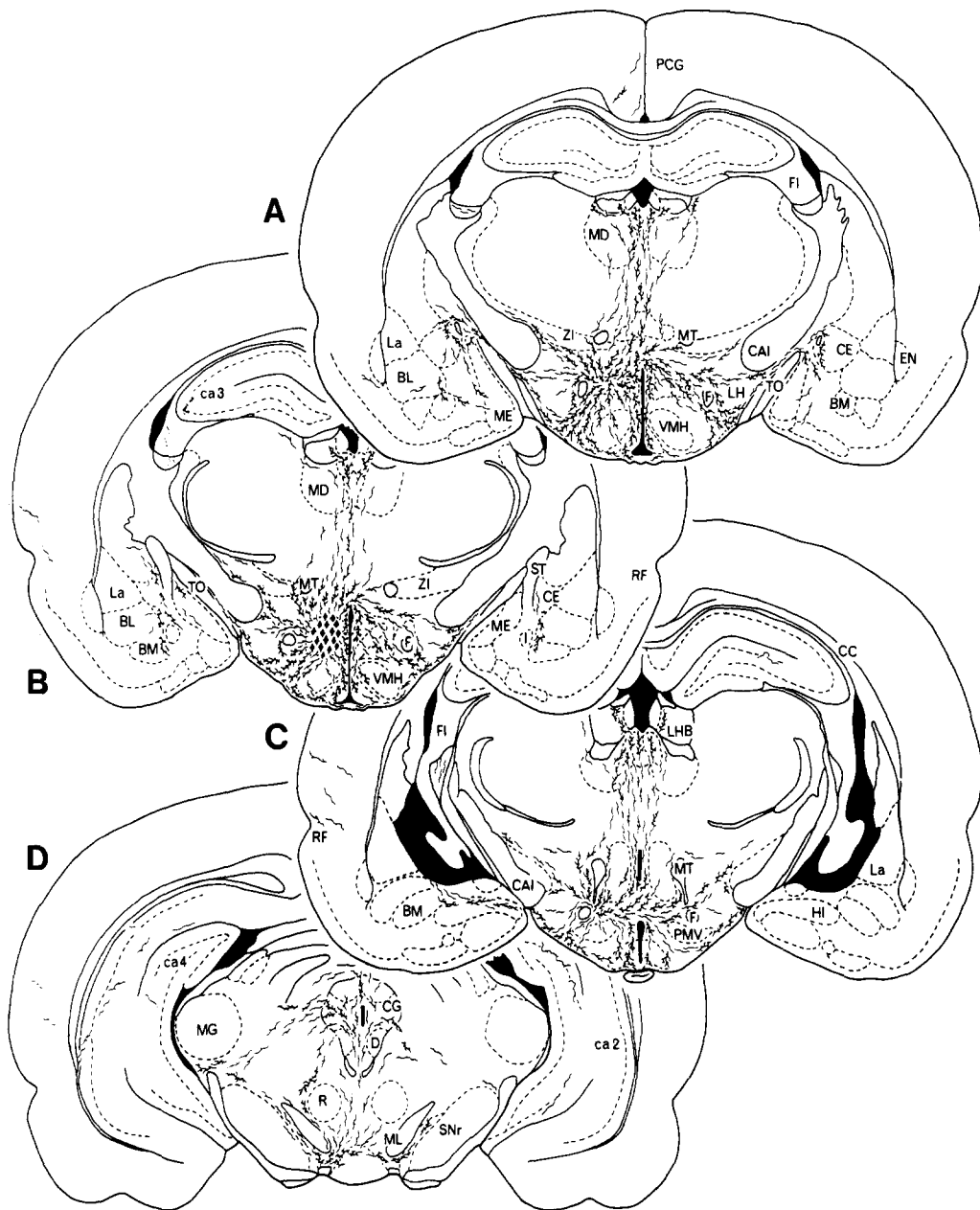


Fig. 21.

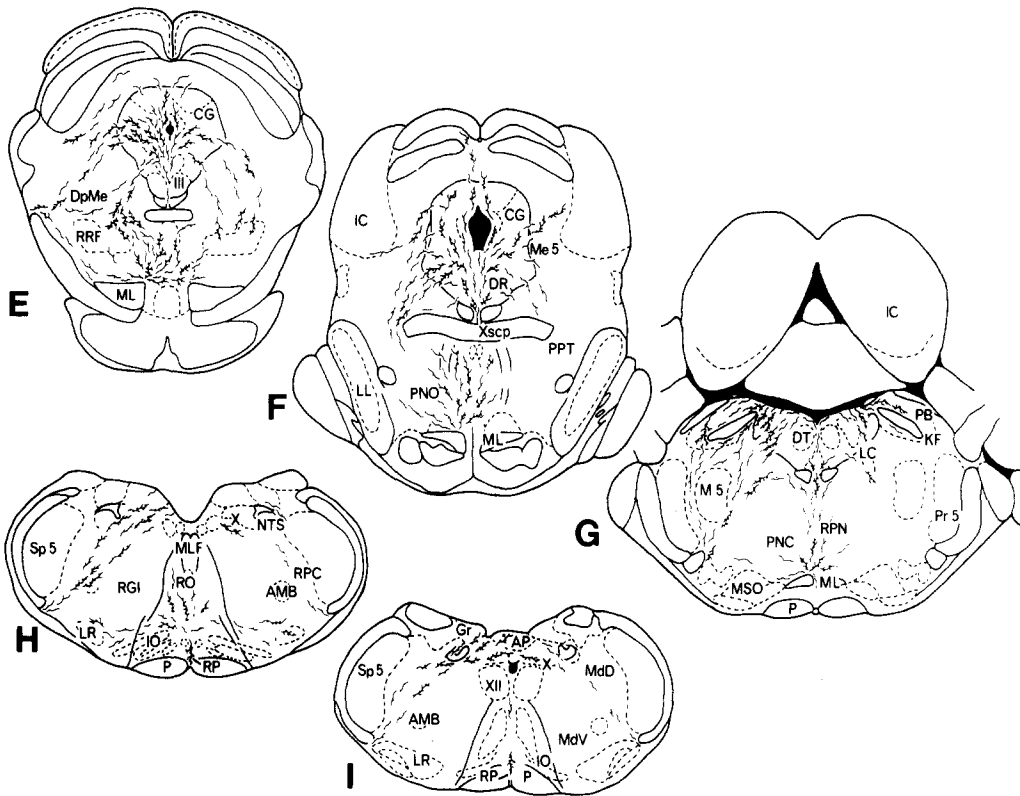
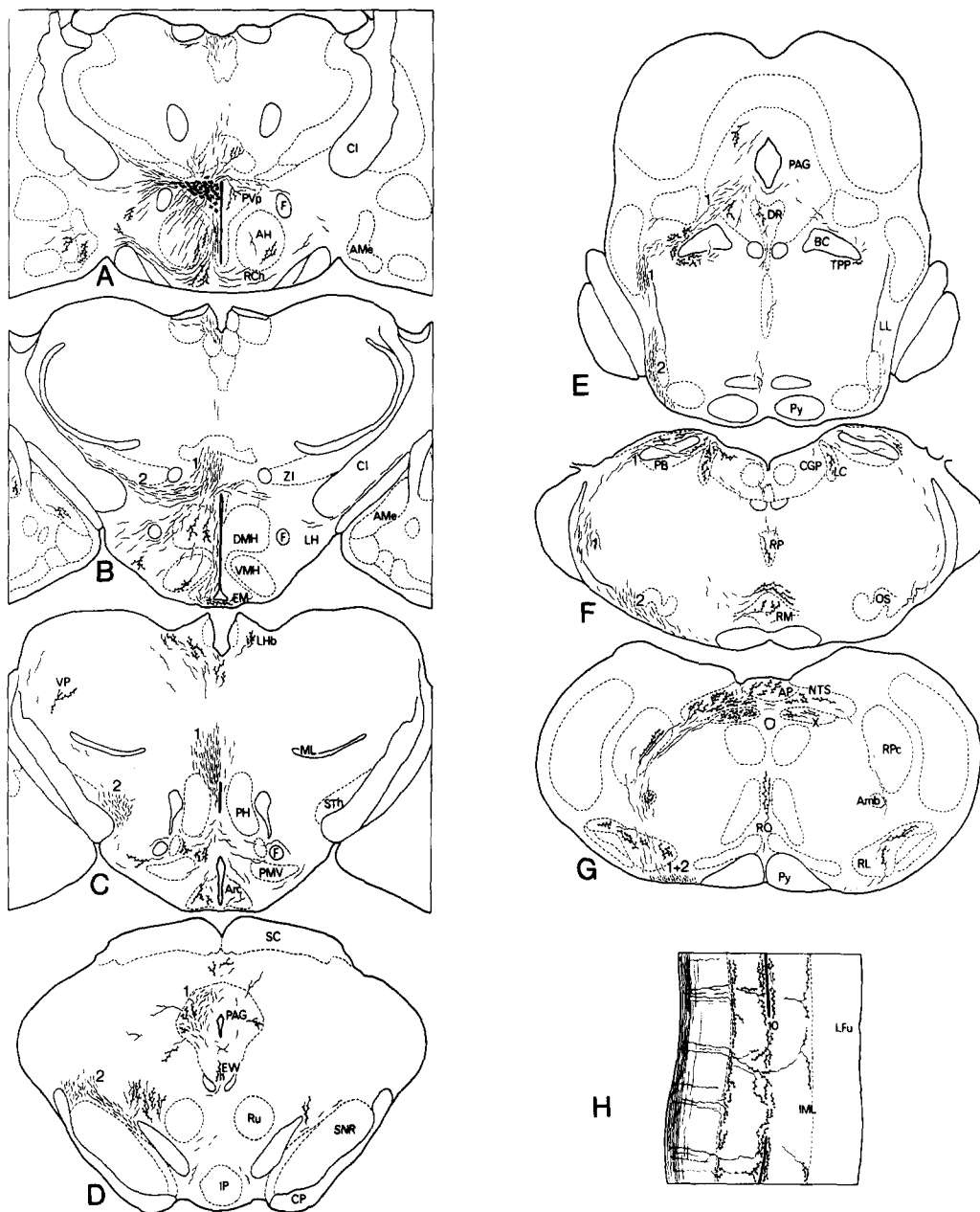


FIG. 21. *Phaseolus vulgaris* leuco-agglutinin injection in the dorsomedial hypothalamic nucleus (B) with anterograde labeling of descending efferent connections from anterior (A) to posterior (I).

mental nucleus and parabrachial nucleus, locus coeruleus and various raphe nuclei. The lower brainstem projections of the PVN are dominated by the highly organized and circumscribed terminations in the AMB and DMV, solitary tract nucleus and area postrema, RPC, ventral reticular nucleus and lateral reticular nucleus (RL). Via AMB and DMV the PVN has a direct and apparently powerful inhibitory influence on the parasympathetic cell groups of the ANS (Rogers and Hermann, 1985; Rogers and Nelson, 1984).

Beyond the lower medulla, an impressive contingent of fibers moves more laterally in the dorso-lateral funiculus. The PVN efferents can be followed over the entire length of the cord giving off fiber collaterals that run up to and heavily branch in the intermediolateral column of the thoraco-lumbar segments. After ending in the IML most fibers continue a medially directed course and produce terminal boutons in area 10 of Rexed which is the midline extension of the IML and will probably comprise sympathetic preganglionic neurons as well (Luiten *et al.*, 1984). Comparing the PHA-L data described here with immunocytochemical tracing of fiber pathways with antibodies against vasopressin (Buijs, 1978; De Vries *et al.*, 1985) and against oxytocin (Swanson and McKellar, 1979), shows a highly remarkable similarity. This indicates that in the PVN projection to the parasympathetic cell groups in the lower medulla the neuropeptide vasopressin acts as a neurotransmitter, whereas in the sympathetic PVN-IML projection a transmitter role may be attributed to oxytocin. In this respect we should emphasize the transmitter function capacity which has been demonstrated for both vasopressin and oxytocin (Bohus *et al.*, 1983; Buijs and van Heerikhuizen, 1982).

In summary, the PVN is the only hypothalamic feeding control nucleus that has a highly developed and circumscribed direct output to the medullary parasympathetic cell groups (likely vasopressinergic) and simultaneously to the spinal sympathetic intermediolateral cell column in the thoraco-lumbar cord (probably oxytocinergic). Apart from these



multifarious autonomic connections, the PVN is strongly linked to the neuroendocrine pathways, as we described in Section 5.

7. Preganglionic Innervation of the Endocrine Pancreas and Adrenal Glands

As we have already indicated when describing the descending autonomic connections of the hypothalamus, the ambiguus and dorsal motor vagus nuclei are considered as the classical parasympathetic motor nuclei, whereas the intermediolateral column is regarded as the source of sympathetic efferents.

In the scope of the present review we have reinvestigated which cell groups in cord and brainstem are concerned with preganglionic innervation of the adrenal glands and of the

TABLE 1. TOTAL AMOUNTS OF LABELED SOMATA IN VARIOUS CNS STRUCTURES IN AVERAGE NUMBERS

Structure	Normal animals (n = 7)	Diabetic animals (n = 7)	Difference	%	Test
DMV (L)	1286 (1697)	945 (1247)	- 341 (450)	- 26.5	S
DMV (R)	729 (962)	658 (868)	- 71 (94)	- 9.8	NS
AMB (L)	101 (133)	54 (71)	- 47 (62)	- 46.6	S
AMB (R)	108 (142)	55 (73)	- 54 (69)	- 48.6	S
C ₃ -C ₄ (L)	243 (214)	100 (88)	- 143 (126)	- 58.8	S
C ₃ -C ₄ (R)	263 (232)	121 (107)	- 142 (125)	- 53.8	S
T ₃ -L ₂ (L)	757 (863)	856 (976)	+ 99 (113)	+ 13.0	NS
T ₃ -L ₂ (R)	772 (880)	805 (918)	+ 33 (38)	+ 4.9	NS

Given are the mean numbers of cells counted in every second section. In parentheses the numbers of cells calculated with corrections for double-counting. Differences were statistically tested with the Mann-Whitney U-test. $P < 0.05$ was considered significant (S), larger values non-significant (NS).

endocrine components of the pancreas by means of retrograde labeling of the nerve cells after tracer injections in the target organs. The pattern of labeling that appears after HRP injections in the adrenal glands is basically simple. For a considerable part the labeling confirms previous reports by Ross *et al.* (1984) and Schramm *et al.* (1976), and consist of a clearly labeled longitudinal line of cells in the IML that is strictly ipsilateral to the injection site. It is worth mentioning that no sympathetic adrenal preganglionic somata are present in the intermedio-medial column for area 10 of Rexed. On the other hand, we found a hitherto unreported population of labeled somata in the ipsilateral ventral horns of cervical segments 3 and 4. There are reasons to believe that these large C₃-C₄ neurons fulfil a parasympathetic function in the vascular innervation of the adrenal glands, and possibly of the visceral endocrine organs in general, which will be argued below.

The preganglionic innervation of the islets of Langerhans in the pancreas is considerably more complicated (Laughton and Powley, 1979; Luiten *et al.*, 1984; Weaver, 1980). The innervation of the entire pancreas was revealed by HRP injection within this organ, after which bilateral labeling of cells occurred in the ambiguus and dorsal motor vagus nuclei, in the ventral horn of cervical segments C₃-C₄, in the intermedio-lateral and intermedio-medial column of thoracic segment T₃ to lumbar segment L₂ and in the ventral horn of T₃-L₂ (Luiten *et al.*, 1984) (Table 1). The cells that specifically innervate the pancreatic β -cell were identified by comparing the innervation of the normal pancreas with the innervation of the alloxan-diabetic pancreas. For this comparison, we assumed the entire elimination of β -cells and their innervation by pretreatment with the diabetogenic agent alloxan (Table 1). This reduction—indicative for the position and amount of parasympathetic preganglionic cells—appeared particularly strong in the left DMV, the AMB bilaterally, and the C₃-C₄ cells in the spinal cord. No change in the number of labeled cells after alloxan pretreatment was seen in the sympathetic thoraco-lumbar spinal cord.

These data obtained with the HRP method led us to the following conclusions. Apart from the classical parasympathetic nuclei in the medulla oblongata, there is an additional group of large, probably parasympathetic, neurons in the ventral motor horns of segments C₃-C₄. Secondly, the labeled neurons in the thoraco-lumbar cord are to be considered as being sympathetic in nature both because of their position in the IML and IMM, and because of their resistance against alloxan treatment, a drug that is known to selectively destroy the parasympathetically innervated β -cell. Both conclusions may seem remarkable. A C₃-C₄ group of large motor neurons innervating endocrine organs like the pancreas and adrenal glands, and also being possibly parasympathetic in nature, has never before been reported and would throw a new light on certain aspects of autonomic innervation of the viscera. An indication of the function of the C₃-C₄ neurons in the innervation of adrenal glands and pancreas is given by the rather specific anatomical organization of the descending connection from the reticular formation to the C₃-C₄ motor column. From our PHA-L experiments in the lower medulla, it became apparent that, in particular, the ventral medullary reticular nucleus (MDV) is a donor of efferents to the C₃-C₄ cell groups

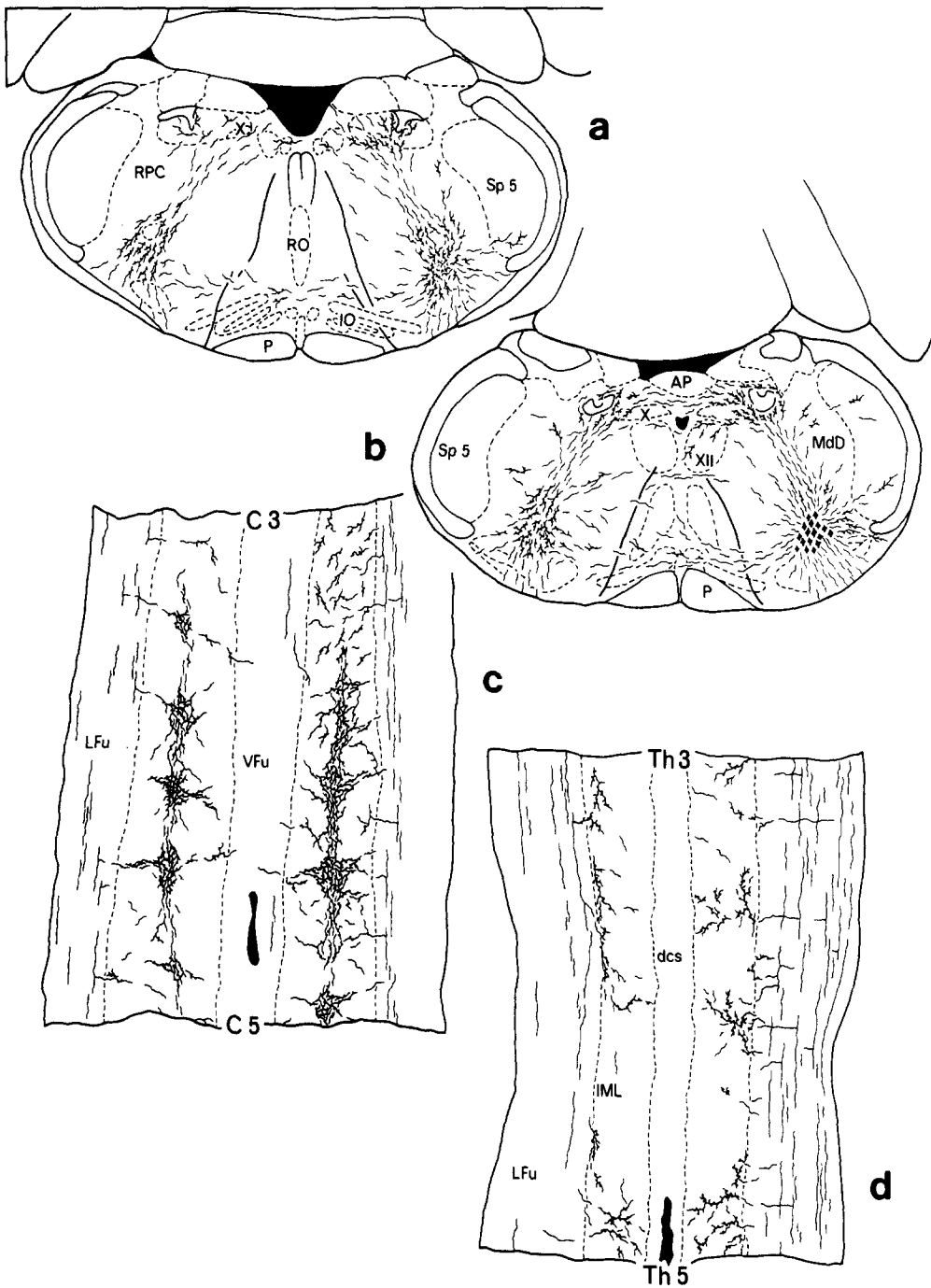


FIG. 23. Spinal efferent projections from the ventral medullary reticular nucleus demonstrated by anterograde transport of PHA-L. Note the injection locus in (b) and the well-organized segmental projection to the motor column of the ventral cervical cord. Compare with Fig. 24.

that innervate the viscera (Fig. 23). The direct anatomical link between the MDV and C₃–C₄ was demonstrated in double labeling experiments in which we injected HRP into the pancreas and PHA-L into the MDV. In such experiments anterogradely labeled MDV efferent terminal boutons are found on motor neurons in C₃–C₄ that are retrogradely labeled with HRP (Fig. 24). Because of the fact that many studies indicate a vasomotor control function for the ventral medulla oblongata (Caverson *et al.*, 1983; Dampney *et al.*, 1982; Day *et al.*, 1983; Ross *et al.*, 1984), we believe that the C₃–C₄ preganglionic cell group may be concerned with the vasomotor control of endocrine visceral organs. Such a well

organized control system, specifically for endocrine organs may prove to be of great importance for the entry of hormones in the general circulation.

The second conclusion we had drawn from our HRP experiments was that a considerable contingent of cells in the sympathetic domain of the spinal cord became labeled after injection of HRP into the pancreas. Such a labeling of sympathetic preganglionic somata in the cord is remarkable, since it does not match the classical concept of sympathetic organization. In this classical view the sympathetic preganglionic neuron synapses on the noradrenergic postganglionic cell in the extramural celiac ganglion. As such the preganglionic cell is not supposed to become labeled after intramural application of HRP, in this case in the pancreas, since HRP is not able to be transferred from postganglionic soma to preganglionic presynaptic boutons. This labeling in the sympathetic cord after HRP injection in the pancreas may be indicative for a direct sympathetic innervation of the pancreas without an extramural ganglionic relay. Consequently, this would imply the presence of postganglionic noradrenergic somata within the pancreatic tissue. Indeed, in a recent combined histochemical and immunocytochemical study, we were able to demonstrate a well-developed pattern of monoaminergic cell bodies in the pancreas that were noradrenergic in nature (Luiten *et al.*, 1986). Direct anatomical relations between descending pathways that originate from the parvocellular reticular formation to sympathetic preganglionic cell bodies in IML and IMM, were demonstrated in combined tracing experiments. In these experiments PHA-L was injected into the RPC and HRP either in the adrenal glands or in the pancreas. In such combined experiments PHA-L labeled terminal boutons could be visualized on or close to retrogradely labeled cell bodies in IML or IMM (Fig. 24).

In conclusion, we have described in this section the location of preganglionic nerve cells that innervate the pancreas and also specifically its endocrine component, and the preganglionic neurons that innervate the adrenal glands. Sympathetic innervation of both visceral targets originate from the intermedio-lateral and, for the pancreas, also from the intermedio-medial cell columns of the thoraco-lumbar cord. Parasympathetic preganglionic cells to innervate the endocrine pancreas are localized in the AMB-DMV complex. A second presumably parasympathetic source of preganglionic neurons could be demonstrated in C₃-C₄ ventral horn segments. The latter cell groups possibly participate in vasomotor control of the visceral endocrine organs. Connections between the medullary reticular formation to the above-mentioned autonomic cell groups were identified in combined anterograde and retrograde tracing experiments.

8. Summary and Conclusions

The hypothalamic output pathways that underlie release of hormones in metabolic regulation have been summarized in Figs 25 and 26. In Fig. 25 a diagrammatic survey is given of the outgoing connections to the autonomic nervous system, that originate in the ventromedial, lateral and dorsomedial hypothalamic nuclei.

The mainstream of descending output from the ventromedial nucleus (VM in Fig. 25) is aimed at the periaqueductal gray from where connections are directed at the ventrolateral and ventromedial portions of the medullary reticular formation (RET in Fig. 25). From these reticular areas in the lower brainstem projections could be demonstrated to sympathetic preganglionic somata in the intermediolateral cell column in the thoracolumbar cord (IML). The IML not only consists of somata that maintain the sympathetic innervation of the gastro-intestinal channel and related organs such as the pancreas. It also contains the preganglionic cell groups that innervate the adrenal medulla. Because of the prevailing physiological effects elicited from the ventromedial hypothalamic nucleus and the nature of the connectivity, we have labeled the above-mentioned output channel as a preponderant orthosympathetic pathway.

The outgoing connections of the lateral hypothalamic area (LH in Fig. 25) for a major part are directly aimed at the parasympathetic cell groups of the vagus/ambiguus complex and at the nearby medullary reticular cell groups that give rise to parasympathetic

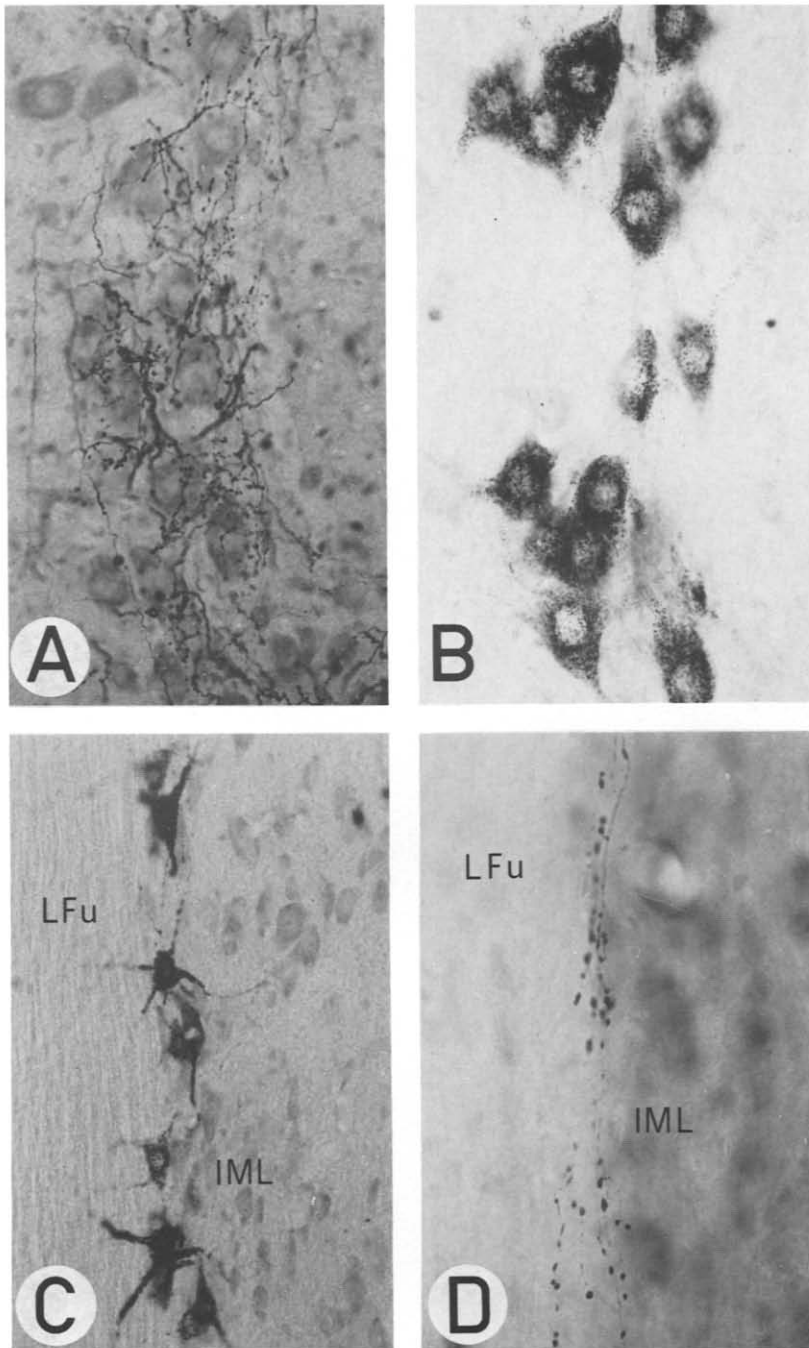


FIG. 24. (A) Photomicrograph of anterogradely PHA-L labeled terminations in the ventral horn of the cervical cord after a tracer injection in the ventral medullary reticular nucleus. Compare with Fig. 23c. (B) Horizontal section at the same cervical cord level showing the retrograde labeling of cell bodies after an HRP injection in the pancreas. (C) HRP labeled somata in the intermediolateral column resulting from an HRP injection in the adrenal gland. (D) Photomicrograph of a PHA-L preparation at the same thoracic segments as in photograph C, showing anterogradely labeled presynaptic boutons in the IML following a PHA-L injection in the medullary parvocellular reticular formation.

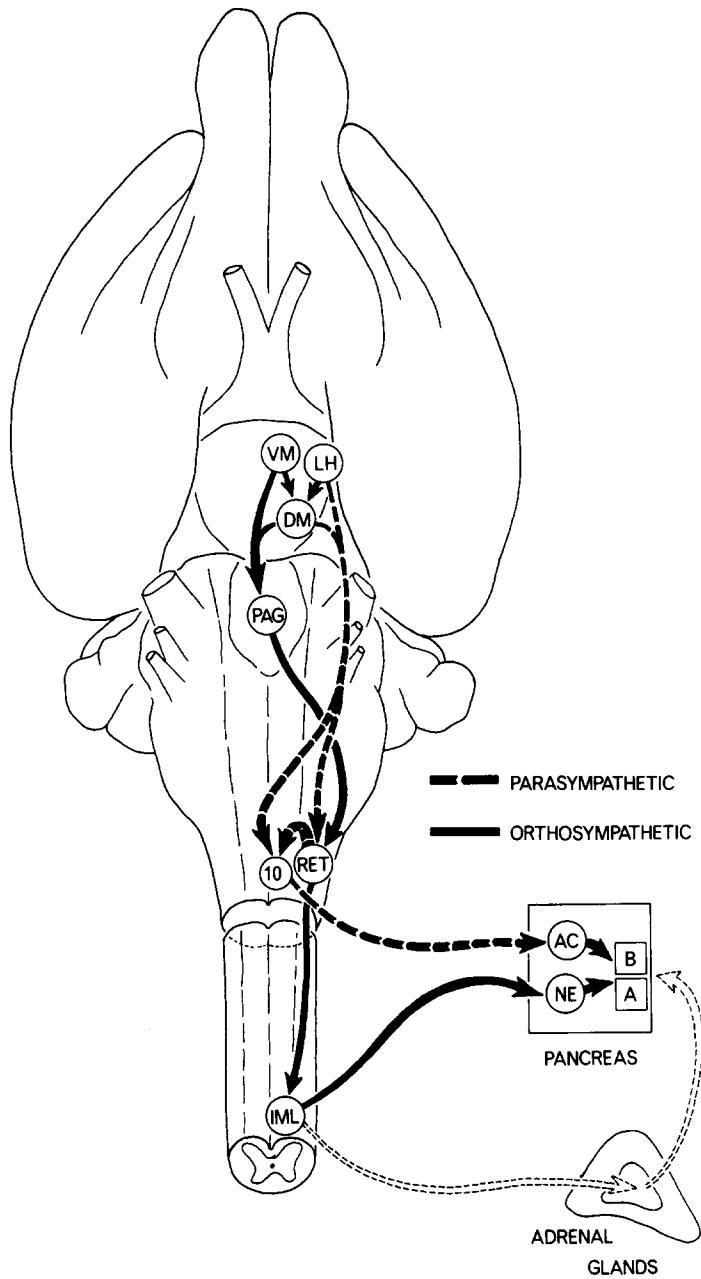


FIG. 25. Diagrammatic representation of the descending sympathetic and parasympathetic pathways originating from the lateral (LH), ventromedial (VM) and dorsomedial (DM) hypothalamic nuclei. Both direct and indirect pathways are discerned. Direct connectivity from LH and DM is aimed at parasympathetic cell groups in dorsal motor vagus and ambiguus nuclei (10). Indirect circuitry includes major relay centers in periaqueductal gray (PAG) and lower medullary reticular formation (RET) predominantly aimed at the sympathetic preganglionic somata in the intermediolateral column.

projections as well. The dorsal motor vagus and ambiguus nuclei harbour the parasympathetic, preganglionic nerve cells that supply efferents to the pancreas and to the entire visceral tissue mass. The direct LH-medulla connection should be considered as the dominant parasympathetic hypothalamic outflow pathway. LH projections to the periaqueductal gray (not indicated in the diagram in Fig. 25), however, open the possibility for sympathetic signals from the LH to reach their visceral target organs via the main VM-PAG-RET-IML circuit.

The dorsomedial hypothalamic nucleus (DM in Fig. 25) in our opinion plays an

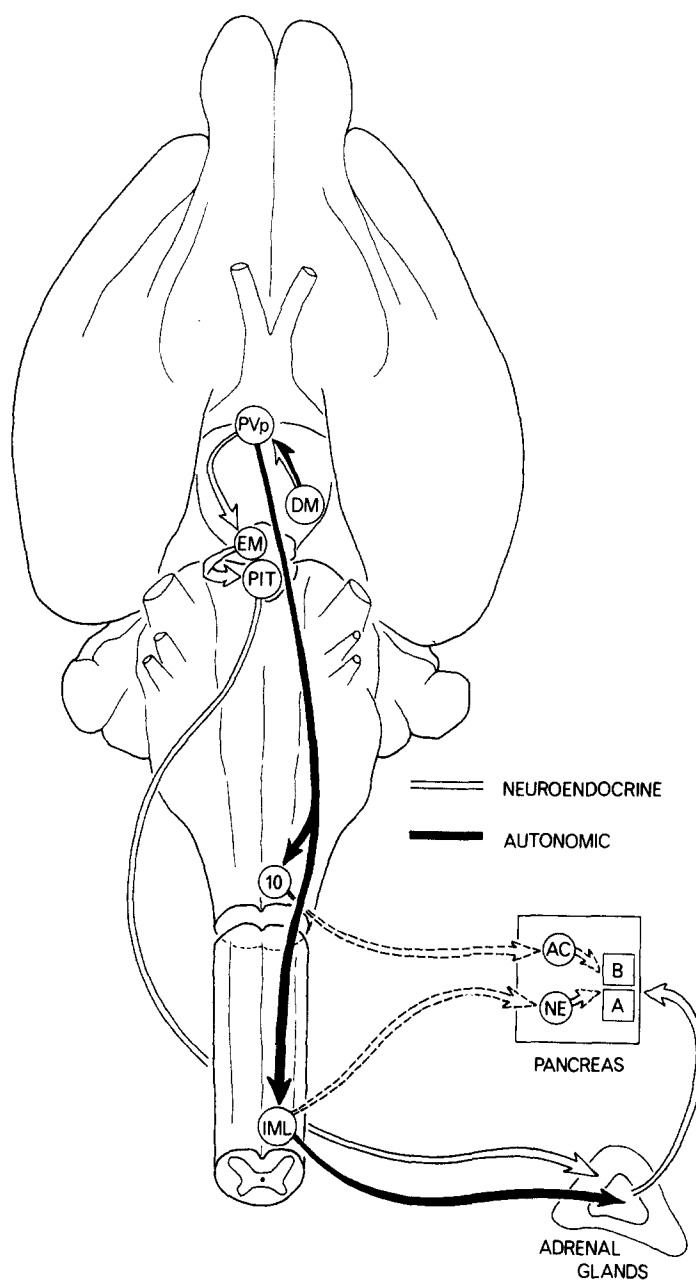


FIG. 26. Diagrammatic survey of outflow channels of the paraventricular nucleus (PVP). Black arrows indicate neural autonomic connections to the various sympathetic and parasympathetic cell groups that innervate gastro-intestinal tract and related organs (here represented by the pancreas and the adrenal medulla). The neuro-endocrine connections to the adrenal cortex are indicated by open arrows including releasing factor and hormonal circuits via the median eminence and the posterior pituitary.

important role in intrahypothalamic integration, but gives rise to descending output connections as well. In terms of autonomic characterization, the DM takes an intermediary position between VM and LH. The DM receives a considerable input from both the ventromedial and lateral hypothalamus. Moreover, it possesses presumably sympathetic projections to the PAG, and parasympathetic connections to the dorsal motor vagus nucleus. Within the hypothalamus, the remarkably well-developed DM projections to the paraventricular nucleus is the most striking feature of the DM in relation to the control of feeding and metabolism.

The output pathways of the paraventricular hypothalamic nucleus to the endocrine

system are two-fold; autonomic and neuroendocrine (Fig. 26). The neuroendocrine pathway originates from cell groups in the parvocellular paraventricular nucleus that are responsible for the release of corticotropin releasing-factor (CRF) in the portal vasculature of the median eminence (EM). Blood-borne CRF then stimulates the release of adrenocorticotrophic hormone (ACTH) from the adenohypophysis (PIT in Fig. 26), which regulates the mobilization of corticosteroids from the adrenal cortex. Corticosterone affects glucose homeostatic mechanisms by its influence on the sensitivity of glucose of the pancreas β -cell.

The autonomic connections of the paraventricular nucleus are both uncomplicated and unique. Presumably vasopressinergic and oxytocinergic cell groups in the PVP give rise to circumscribed axonal pathways to all autonomic preganglionic cell groups in brainstem and spinal cord.

Apart from several anatomical sidelines, that we have described in the previous sections, the mainstream of hypothalamic output to the endocrine system does not appear to be overcomplicated. We should realize, however, that we are dealing here with a gross-anatomical pathways analysis. Although such anatomical knowledge to a certain degree may indicate the functional nature of such pathways, we are still far from a synthesis on a structural-functional basis. A large number of investigations, specifically carried out on hypothalamic structures but also on medullary levels, throw some light on the functional significance of the pathways we described.

It is tempting to assume that the effects of electrical and chemical stimulation of the ventromedial and lateral hypothalamic areas on pancreatic and adrenal hormone release is mediated by the autonomic circuits indicated in Fig. 25. These assumptions are strongly substantiated by the absence of increased insulin release by LHA stimulation after application of atropine, indicative of parasympathetic mediation. Involvement of sympathetic circuits in glucagon release becomes evident from the lack of effect of VMH stimulation after application of hexamethonium, a peripheral noradrenergic blocker. The more general autonomic role of the dorsomedial hypothalamic nucleus in pancreatic hormone release is indicated by the effects of DMH lesions (Moltz *et al.*, 1984). These authors described increased insulin levels and simultaneous changes in levels of glucagon as a result of DMH damage.

Stimulation studies on the parasympathetic centers in the lower medulla unequivocally demonstrate the dorsal motor vagus and ambiguus nuclei as being primarily involved in insulin secretion (Bereiter *et al.*, 1981; Bergman and Miller, 1973; Ionescu *et al.*, 1983). Bereiter *et al.* (1981) moreover, demonstrate the importance of the ambiguus surrounding reticular formation in pancreatic innervation. They not only revealed the role of this reticular area in the release of insulin by parasympathetic mechanisms. Apparent disinhibition of this insulin release mechanism was shown by application of the α -adrenergic antagonist phentolamine. This disinhibition suggests a potent role for the ventromedial and ventrolateral reticular formation in sympathetic mechanisms, likely via the RET-IML pathways we described in this survey.

A hitherto unnoticed brain area in control mechanisms for feeding and metabolism is the periaqueductal gray, which we have attributed an important position in the sympathetic hypothalamic output. So far a preliminary exploration has been made of the effects of PAG stimulation on the activity of vagus and splanchnic nerve branches to pancreas and adrenal gland (Fig. 27). PAG was stimulated bilaterally at various coordinates, both electrically and by chemical stimulation with glutamate. In Fig. 27 the effects of repeated electrical PAG stimulation is given by the strongly increased whole nerve activity of the fine branches that innervate the pancreas and the adrenal glands. The relatively short latencies of approximately 8 msec are indicative of a well-organized and rather short connectivity circuit between PAG and the peripheral nerves before entering the target organs. The narrow stimulation activity peaks suggest either synchronization and/or a homogeneous organization of intracerebral pathways in line with our anatomical data.

The neurophysiological experiments described here are exemplary for the impressive complexity of hypothalamic output mechanisms in homeostatic and metabolic regulatory

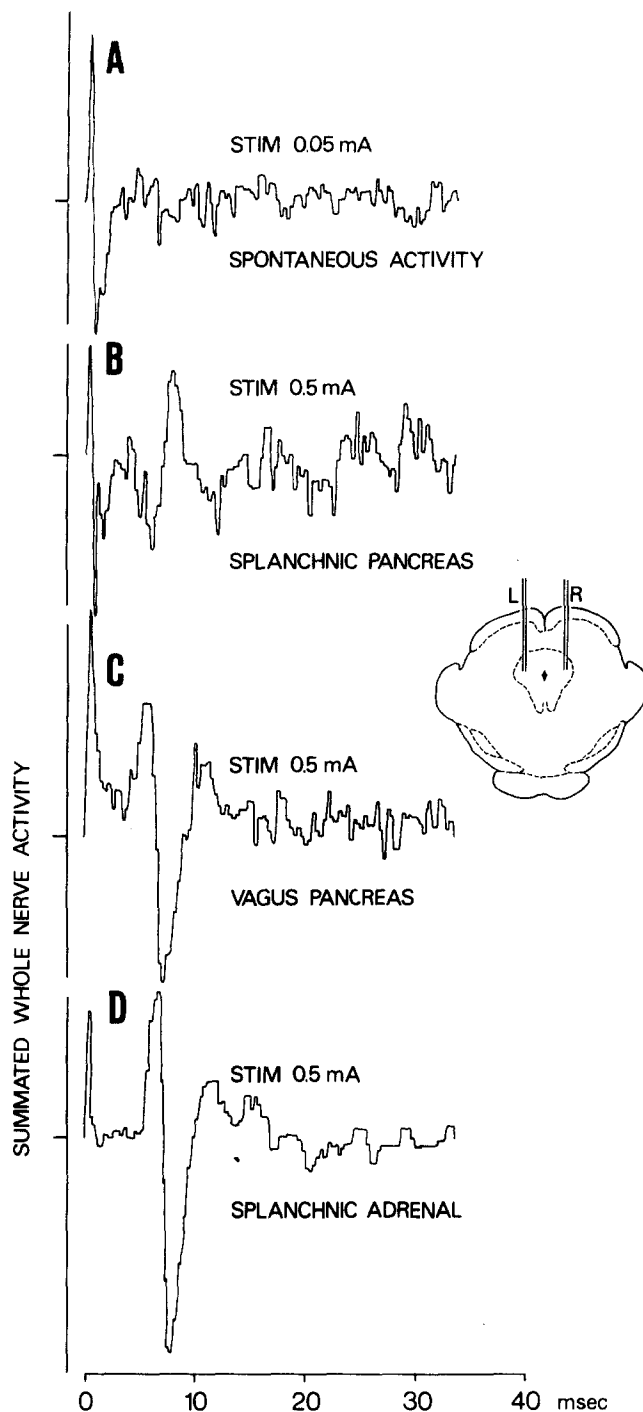


FIG. 27. Summated whole nerve branch recordings of the vagus pancreas nerve and splanchnic pancreas and adrenal nerves after bilateral periaqueductal gray stimulation. Increased peak activity can be observed in these nerves approximately 8 msec after stimulation.

mechanisms on the level of individual neuron activity. In our efforts to gain understanding how the brain is able to control and regulate the body's metabolic functions, our future analysis to a substantial degree will require a thorough neurophysiological approach.

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